ABI 433A Peptide Synthesizer

Installation Guide

Applied Biosystems

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Introduction and Safety 1

Overview

About This Chapter

This chapter contains an introduction to the guide, general safety information and specific chemical hazard warnings for chemicals in the Install Chemistry Kit. Also included are directions for ordering Material Safety Data Sheets (MSDSs).

In This Chapter

This chapter covers the following topics:

Topic	See Page
Introduction	1-2
Safety	1-2

Introduction

About this Guide

The instructions presented in this guide are intended to assist you, a trained Applied Biosystems service engineer, in the installation of an ABI 433A Peptide Synthesizer and the verification of its performance. This guide also serves as a reference at the installation site. For detailed understanding of the instrument, read the *ABI 433A Peptide Synthesizer User Guide* (PN 904855).

This guide is intended for service personnel only who have been specifically trained by Applied Biosystems. Applied Biosystems is not liable for damage or injury that results from use of this guide by unauthorized or untrained parties.

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

⚠ CAUTION Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

⚠ WARNING Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

⚠ DANGER Indicates an imminently hazardous situation which, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

⚠ WARNING CHEMICAL HAZARD. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazard Warning

⚠ WARNING CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Handle chemical wastes in a fume hood.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Chemical Warnings

Hazardous chemicals usedin the installation of the ABI 433A Peptide Synthesizer are listed below with their corresponding chemical hazard warnings.

⚠ WARNING Some of the chemicals referred to in this protocol may not have been provided with your kit. If the chemicals are not provided, they are not manufactured or sold by Applied Biosystems. Please obtain the material safety data sheets from their manufacturers.

Chemicals and Chemical Hazards

Chemical	Chemical Hazard
DCM Dichloromethane	**⚠ CAUTION CHEMICAL HAZARD**. **Dichloromethane (DCM)** may cause eye, skin, and respiratory tract irritation. Exposure may cause central nervous system depression and blood damage. It is a potential human carcinogen. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
DIEA Diisopropylethylamine	**⚠ WARNING CHEMICAL HAZARD**. **Diisopropylethylamine (DIEA)** is a flammable liquid and vapor. Exposure can cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
HBTU *N*-[(*H*-benzotrizol-1-yl) (dimethylamino)methylene]-*N*-methylanaminium hexafluorophosphate *N*-oxide	**⚠ WARNING CHEMICAL HAZARD**. **(*N*-[1*H*-benzotrizol-1-yl) (dimethylamino)methylene]-*N*-methylanaminium hexafluorophosphate *N*-oxide (HBTU)**, formerly 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uroniumhexafluorophosphate, may cause allergic respiratory and skin reactions. Do not breathe the dust, and avoid prolonged or repeated contact with the skin. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Chemicals and Chemical Hazards *(continued)*

Chemical	Chemical Hazard
HOBt Hydroxybenzotriazole	**⚠ WARNING CHEMICAL HAZARD**. **1-Hydroxybenzotriazole hydrate (HOBT)** has a risk of explosion if heated under confinement. Keep away from heat and flame. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
MeOH Methanol	**⚠ WARNING CHEMICAL HAZARD**. **Methanol** is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, and central nervous system depression and blindness. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
NMP *N*-Methylpyrrolidone	**⚠ WARNING CHEMICAL HAZARD**. ***N*-Methylpyrrolidone (NMP)** may cause eye, skin, and respiratory tract irritation. It may adversely affect the developing fetus. It is a combustible liquid and vapor. Keep away from heat, sparks, and flame. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Piperidine hexahydropyridine	**⚠ WARNING CHEMICAL HAZARD**. **Piperidine (hexahydropyridine)** **Note** Piperidine must be ordered by the customer, and it is not provided by Applied Biosystems in the chemical installation kit. Please obtain the material safety data sheet from the manufacturer.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

Obtaining MSDSs

⚠ WARNING CHEMICAL HAZARD. Some of the chemicals provided in your reagent kit may be hazardous. Before handling the reagents, read the material safety data sheets (MSDSs) that accompany your first shipment. Always follow the safety precautions (wearing appropriate protective eyewear, clothing, and gloves, etc.) presented in each MSDS. To receive additional copies of MSDSs at no extra cost, use the contact information below.

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

Obtaining MSDSs

Step	Action
1	Go to https://docs.appliedbiosystems.com/msdssearch.html
2	In the Search field of the MSDS Search page: a. Type in the chemical name, part number, or other information that you expect to appear in the MSDS of interest. b. Select the language of your choice. c. Click **Search**.

Obtaining MSDSs *(continued)*

Step	Action
3	To view, download, or print the document of interest: a. Right-click the document title. b. Select: – **Open** – To view the document – **Save Target As** – To download a PDF version of the document to a destination that you choose – **Print Target** – To print the document
4	To have a copy of an MSDS sent by fax or e-mail, in the Search Results page: a. Select **Fax** or **Email** below the document title. b. Click **RETRIEVE DOCUMENTS** at the end of the document list. c. Enter the required information. d. Click **View/Deliver Selected Documents Now**.

Note For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Instrument Safety Labels

Safety labels are located on the instrument. Each safety label has three parts:

- A signal word panel, which implies a particular level of observation or action (*e.g.,* CAUTION or WARNING). If a safety label encompasses multiple hazards, the signal word corresponding to the greatest hazard is used.
- A message panel, which explains the hazard and any user action required.
- A safety alert symbol, which indicates a potential personal safety hazard. See the *ABI 433A Peptide Synthesizer Site Preparation and Safety Guide* for an explanation of all the safety alert symbols provided in several languages.

About Waste Disposal

As the generator of potentially hazardous waste, it is your responsibility to perform the actions listed below.

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, or national regulations.

Note Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Moving and Lifting the Instrument

⚠ CAUTION PHYSICAL INJURY HAZARD. Improper lifting can cause painful and sometimes permanent back injury.

Use proper lifting techniques when lifting or moving the instrument. Safety training for proper lifting techniques is recommended.

Do not attempt to lift or move the instrument without the assistance of others. Depending on the weight of the instrument, this action may require two or more people.

Before Operating the Instrument

Ensure that everyone involved with the operation of the instrument has:

- Received instruction in general safety practices for laboratories
- Received instruction in specific safety practices for the instrument
- Read and understood all related MSDSs

⚠ CAUTION Avoid using this instrument in a manner not specified by Applied Biosystems. Although the instrument has been designed to protect the user, this protection can be impaired if the instrument is used improperly.

Preinstallation Preparation 2

Overview

About This Chapter

This chapter contains general information about preinstallation preparation for the ABI 433A Peptide Synthesizer. Preinstallation preparation includes verifying that all equipment and reagents are present, and verifying that all requirements on the preinstallation checklist are met. Uncrating the instrument and connecting the power to the instrument, the computer and the computer monitor completes the preinstallation preparation.

In This Chapter

This chapter covers the following topics:

Topic	See Page
Verifying the Receipt of Materials	2-2
ABI 433A Preinstallation Checklist	2-3
Uncrating the Instrument	2-5
Preparing for Instrument Installation	2-6
Input/Output Connections	2-7

Verifying the Receipt of Materials

Preinstallation Checklist and the Site Preparation and Safety Guide

Before the installation date, the instrument operator should have received the Preinstallation Checklist and the *ABI 433A Peptide Synthesizer Site Preparation and Safety Guide* (PN 902475), which includes a checklist for preparing the laboratory. Ensure that each item on the checklist has been completed before beginning the installation procedure. For convenience, a checklist is included in this section.

FastMoc™ Reagents

The Install Chemistry Kit is the *FastMoc*™ Kit (PN 401465). The Install Chemistry Kit, containing installation *FastMoc*™ reagents and chemicals, is shipped separately. The startup chemicals should be unpacked immediately upon arrival and stored according to labelled directions. Verify the contents of the reagent and chemical kit prior to starting the installation.

⚠ WARNING CHEMICAL HAZARD. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (e.g., fume hood). For additional safety guidelines, consult the MSDS.
- Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.

Ordering Piperidine

Your customer needs to have piperidine in the laboratory. Customers may need to contact Sigma-Aldrich to register and purchase piperidine. To register and place orders from anywhere in the world, customers can go to:

http://www.sigmaaldrich.com

To place orders from Europe, the Middle East, or Africa, customers can also go to:

http://sigmaaldrich.com/Europe

The Aldrich part number is 57,126-1 for 200- and 450-mL sizes of piperidine.

Note Aldrich provides one part number for several sizes of piperidine, including the 200- and 450-mL size bottles.

ABI 433A Preinstallation Checklist

Using the Checklist

Please review this checklist carefully, marking all delivered, completed, or "ready" items. References indicate the appropriate sections in the *ABI 433A Peptide Synthesizer Site Preparation and Safety Guide.*

Preinstallation Checklist

Customer Information

Item	Description
Organization name	
User's name	
Lab address	
Phone number	

ABI 433A Instrument Serial Number, Shipping and Installation Dates

Item	Description
Serial number	
Scheduled ship date	
Installation date	
Support engineer	

Site Preparation Checklist

✔	Date Confirmed	Requirement
		ABI 433A Peptide Synthesizer Site Preparation and Safety Guide received and read.
		Sufficient lab space per site preparation and safety guide.
		Correct electrical power per site preparation and safety guide.
		Electrical receptacles located within 5 m (15 ft) of the rear panel of the instrument (3 receptacles minimum, 4th for printer option).
		Lab temperature 18 to 25 °C, controlled to ±3 degrees.
		Gas cylinder of high purity nitrogen.
		CGA580 two-stage gas regulator (~65 psi output) with needle valve shut off.
		Safety device for securing gas cylinder.
		Correct ventilation and fume hood requirement met as described in "Laboratory Ventilation Requirements" in the site preparation and safety guide.
		Liquid waste secondary containment vessel available.
		Install Chemistry Kit received and properly stored: ♦ *FastMoc*™ Kit (PN 401465) or ♦ UV Chemical Install Kit (PN 4335884)
		Aldrich Piperidine present in the laboratory.

Site Preparation Checklist *(continued)*

✔	Date Confirmed	Requirement
		Material Safety Data Sheets and waste profiles in the site preparation and safety guide read.
		Waste disposal provisions made.
		ABI 433A instrument received.

Uncrating the Instrument

Inspecting the Crate and Uncrating the Instrument

The ABI 433A Peptide Synthesizer is shipped in a single wooden crate that also contains the *ABI 433A Peptide Synthesizer User Guide* (PN 904855), SynthAssist 3.1 software and guide (PN 4344181), parts, and accessories. In addition, the computer system is shipped in two boxes.

The accessories are in the top compartment of the crate, which can be accessed by removing the spring clips with the claw end of a hammer. As noted previously, the installation reagents are shipped separately and should be checked for completeness on site before you begin the installation.

To inspect and uncrate the instrument:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Inspect the shipping containers for damage before unpacking them.

<table><tr><th>If...</th><th>Then...</th></tr><tr><td>damage is evident</td><td>determine whether damage occurred
♦ During shipment
♦ At the customer site</td></tr><tr><td>damage appears to have occurred during shipment</td><td>retain all packing material and contact Applied Biosystems Traffic Department for instructions on filing a claim</td></tr></table></td></tr>
<tr><td>2</td><td>Unpack the shipping container.</td></tr>
<tr><td>3</td><td>Check the contents of the container against the packing list.</td></tr>
<tr><td>4</td><td>Verify that you have all items on the packing list.

Note Accessories are packed in the TOP of the shipping crate; do not discard the shipping crate until you have verified that you have ALL items on the packing list.</td></tr>
<tr><td>5</td><td>Remove the instrument from the container and place the instrument where it is to be installed.

⚠ WARNING PHYSICAL INJURY HAZARD. Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. Two or three people are required to lift the instrument, depending upon instrument weight.</td></tr>
<tr><td>6</td><td>Place the computer system so that both the instrument and the computer can be physically reached by the installer without additional walking.</td></tr>
<tr><td>7</td><td>Place the computer system within 15 ft of the instrument.

IMPORTANT The instrument is specified to operate at a distance not exceeding 15 feet from the computer system.

Note The supplied cable length is 15 ft.</td></tr>
</table>

Preparing for Instrument Installation

Verifying the Site is Ready for Instrument Installation

To verify the site is ready for instrument installation:

Step	Action
1	Verify the contents of the Installation Reagent and Chemical Kit (PN 401465 or PN 4335884) are present. ♦ The Installation Reagent and Chemical Kit is shipped separately. ♦ The kit should have been unpacked and inspected by the customer before your arrival.
2	Verify that Methanol and Aldrich piperidine (450 mL size) are present in the laboratory. **IMPORTANT** Methanol must be present to run Flow Test 20.
3	Verify that all requirements for installation are satisfied. ♦ The proposed location allows access to a fume hood and enough space to set up one nitrogen cylinder. **IMPORTANT** If you install the instrument with Argon gas, it may affect the chemical delivery rates of the system, generally delivering higher volumes than specified. ♦ The instrument will be installed in a room at normal temperature and away from direct sunlight. ♦ Location provides access to the right side of the instrument (as viewed from the front), and leaves room for any optional user-supplied fraction collector and bottles 9 and 10. **⚠ WARNING PHYSICAL INJURY HAZARD**. Never place a fraction collector on top of the instrument. It is possible for the fraction collector to fall, resulting in injury to personnel, spilled chemicals, damage to equipment, and lost data.

Releasing the Vortexer Assembly from the Shipping Restraints

The vortexer assembly is secured to the instrument during shipment and the restraints must be removed before the instrument can be installed.

To remove the vortexer assembly from the shipping restraints:

Step	Action
1	Open the rear panel of the instrument.
2	Remove and discard the three screws (marked with green stickers) that secure the vortexer assembly to the chassis.
3	Tighten all fittings marked with green tape and discard the tape.

Configuring the Instrument, Vortexer and Computer for Power

The ABI 433A instrument is self-configuring, and the installer need only select the correct power cord to configure it. The vortexer is a DC drive motor and it does not matter whether the input is 50 or 60 Hz; consequently, there is only one drive belt and there are no adjustments to be made to the vortexer to configure it for power.

The ABI 433A instrument is equipped with a computer which is also automatically configured for input power. Connect power to the ABI 433A instrument, the computer, and the computer monitor.

Input/Output Connections

Location

The locations of the input/output connections on the ABI 433A Peptide Synthesizer are shown in Figure 2-1 below. The input/output connections are also labeled on the instrument.

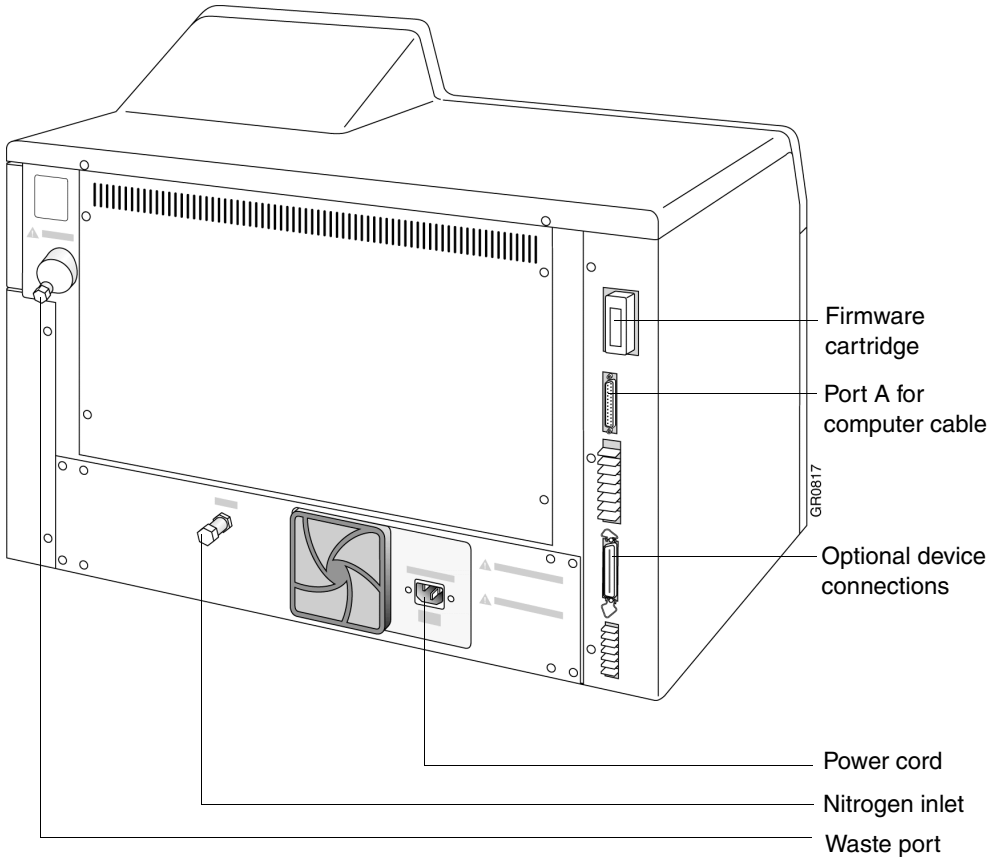


Figure 2-1 Input/output connections on the ABI 433A Peptide Synthesizer

Initial Instrument Installation 3

Overview

About This Chapter

This chapter describes the initial installation of the ABI 433A Peptide Synthesizer. Procedures for connecting the waste collection system to the instrument and to the ventilation system are described, as well as procedures for connecting nitrogen gas to the instrument and testing the instrument for leaks.

Procedures for checking the instrument setup, connecting the computer system to the instrument and performing pressure tests are also described. Turning on the instrument and inspecting the system for proper functioning by running the self-test and calibrating the barcode reader completes the list of procedures included in the initial installation.

In This Chapter

This chapter covers the following topics:

Topic	See Page
Venting and Waste Container Placement	3-2
Connecting Nitrogen Gas to the ABI 433A Instrument	3-4
Leak Test	3-5
Instrument Setup	3-6
Pressure Tests	3-7
Inspecting the System	3-10
Calibration of the Barcode Reader	3-11

Venting and Waste Container Placement

Venting

It is critical both for safety reasons and for the proper operation of the ABI 433A Peptide Synthesizer that the waste and venting systems be properly connected. An incorrectly vented system can result in degraded synthesis and cause damage to the instrument.

IMPORTANT The ABI 433A instrument must be vented according to the venting diagram found in Appendix C.

As noted in the diagram, tubing must be run as straight as possible. There must be no bends or sharp turns that allow creation of a “trap” for condensed fumes. Such a condition can result in a plug of waste material preventing proper venting of fumes. This can contaminate the instrument and cause poor performance.

The waste and venting systems converge at the waste bottle as shown in Figure 3-1. The waste container (PN 140040) is shipped with the instrument. All exhaust gasses and fumes, as well as all expended reagents, are sent to the external bottle. It is the bottle itself that is vented.

Connecting the Waste Line to an External Waste Container

⚠ WARNING CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

The following are guidelines for storing hazardous waste:

- Always use secondary containment when storing chemical waste.
- Store waste for only short periods of time.
- Store only small amounts of waste in the laboratory.
- Store waste away from direct sunlight or sources of heat (on or off the instrument).

The external bottle must be placed at a lower level than the instrument's waste port, which is located at the rear of the instrument. The line from the waste port must slope constantly downward, without sags as these will act as traps. Traps collect reagents or condensed fumes, which impede proper disposal of reagents. Also, traps allow fumes to invade the system, potentially damaging or contaminating components.

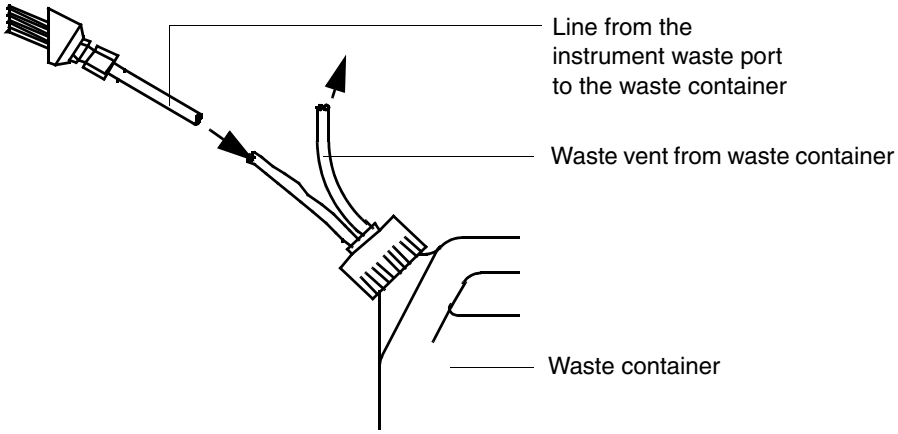


Figure 3-1 Waste Container and Vent Lines

IMPORTANT Place the waste bottle in a secondary container to minimize the danger of leaks.

Locate a convenient place near the instrument to place the waste container. The container must be:

- Easily visible so that it can be checked to be sure it is not full.
- Easily accessible so that it can be emptied easily and safely. (When it's full it will be heavy and it may be difficult to remove the cap if it is not easily accessible.)
- Placed lower than the instrument waste port; the line from the waste port must slope constantly downward, without sags or kinks.
- Placed within a secondary containment vessel so that spills will be contained.

Connecting Nitrogen Gas to the ABI 433A Instrument

Connecting Nitrogen Gas

Nitrogen gas is used to push liquid reagents through the ABI 433A instrument tubing and reagent cartridges. It is also used to dry the interior of the tubing. The following procedure describes how to attach nitrogen gas to the instrument.

⚠ WARNING **EXPLOSION HAZARD**. Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

IMPORTANT If you install the instrument with argon gas, it may affect the chemical delivery rates of the system, generally delivering higher volumes than specified.

To attach nitrogen gas to the instrument:

Step	Action
1	Connect the user-supplied regulator (with CGA 580 adaptor or equivalent) to the nitrogen (99.998% or greater purity) cylinder.
2	Attach the Swagelok™ adapter provided (PN 110005) to the regulator.
3	If necessary, cut the polypropylene tubing (PN 225016) to an appropriate length and ensure that each end is smooth and perpendicular to the sides of the tubing.
4	Use a Swagelok adapter to connect the tubing to the gas cylinder.
5	Blow the tubing clean with the nitrogen before connecting it to the instrument.
6	Remove the red guard cap from the gas input port of the ABI 433A instrument, and attach the other end of the Parflex™ tubing to the port.
7	Adjust the cylinder regulator setting to 0 (zero) psi, and close the valve.
8	Increase the cylinder regulator setting slowly to 65 psi (450 kPa). This is the optimal pressure setting for operating the instrument. **IMPORTANT** Do not exceed an input pressure of 75 psi, as damage to equipment and possible injury to personnel can result.

⚠ WARNING **BOTTLE EXPLOSION HAZARD**. Bottle explosion can cause severe physical injury. To prevent reagent bottles from becoming overpressurized and possibly exploding, make sure that the gas tank pressure does not drop below 300 psi while the instrument is running. If pressure drops below 300 psi, organic solvents can corrode components, backflush into the pressure regulator, and cause regulator failure. Bottle explosion can follow. Do not let the gas tank depressurize during instrument operation.

Leak Test

Testing for Leaks

The instrument must be tested for leaks before chemicals are installed. (Some portions will be leak-tested again, after chemicals are installed.) Testing allows detection and isolation of leaks. It is essential to repair immediately any leaks disclosed, so that leaking solvents or reagents will not come into contact with personnel or the instrument.

Input and Unregulated Internal Pressure Leak Test

This will test the gas supply fittings, the vacuum assist input, both regulators, and one port of each brass cylinder of the autosampler assembly.

Note During these tests the instrument regulators will all read 0 (zero) psi.

To perform the input and unregulated internal pressure leak test:

Step	Action
1	Set the manual valves (“Vent Switches”) for bottles 9 and 10 to the “vent” position (Switch DOWN). **Note** These valves are located to the rear of the right-side panel of the instrument (as viewed from the front). This is referred to as the “plumbing side” of the instrument.
2	With instrument power **off** and the regulator set for 65 psi, close the compressed gas cylinder valve.
3	Turn the knob of the 65-psi regulator several turns counterclockwise on the secondary regulator, and monitor pressure on the main tank. **Note** There will be an initial drop on both the secondary regulator and at the main gauge.
4	Continue to test for at least 5 min. **Note** The pressure drop should not exceed 200 psi per 5 min from the baseline pressure as read on the main gauge.

If the pressure drop...	Then...
does not exceed 200 psi per 5 min	Proceed with the installation.
exceeds 200 psi per 5 min	Recheck all fittings and connections, reset regulator, and repeat this test before proceeding. **IMPORTANT** Do not simply tighten fittings further. Overtightening can cause damage. Instead, open and reseat connections as necessary.

Instrument Setup

Checking the Pusher, Valve Block Fittings and In-Line Filters

The next task is to check the instrument setup. Verify that the pusher, valve block fittings and inline filters are installed correctly.

To check the pusher, valve block fittings and inline filters:

Step	Action
1	Verify the tension and gliding action of the pusher. **Note** Movement should be smooth.
2	Verify that the pusher can be latched and released easily at the left end of the instrument.
3	Verify that the plunger ball alone will hold the pusher, without assistance from the latching mechanism. **Note** Do not allow the block to “snap” to the other end of the instrument.
4	Examine each lock nut on the valve block fittings and verify that they are snug.
5	Check that each inline filter fitting is tight.

Connecting the Computer System to the Instrument

The instrument must be connected to the computer before any additional testing can be performed on the instrument.

To connect the computer to the instrument:

Step	Action
1	Connect the computer system to the instrument: a. Connect one end of the interface cable (PN 4342732) to “Port A”, the RS232 port of the ABI 433A instrument. b. Connect the other end of the interface cable to position 1 of the serial communication port on the back of the computer.
2	Turn instrument power ON, and perform the additional pressure testing and leak checking specified in the following procedures.

Pressure Tests

Instrument Regulators Pressure Test

This test will check both regulators, the gas port of the eight-port valve block, the Angar valves 30, 33, 28, 29 which supply pressure to bottles 1 through 8; and the vent valves (“toggle switches”) for bottles 9 and 10.

To test the instrument regulators pressure:

Step	Action
1	Set the upper regulator to 3 psi.
2	Set the lower regulator to 11 psi.
3	Wait 1 min, then back out both regulators fully. You may see an initial pressure drop while backing out the regulators. **Note** Any pressure drop must be less than 0.30 psi in 3 min.
4	**IMPORTANT** Vent system pressure for the next test. Turn power on to the ABI 433A instrument.
5	Go to the **Main Menu** on the instrument front panel.
6	Select **Manual Control**.
7	Activate function 75 (Gas-Vent2).
8	Using the manual Pressure/Vent switch, vent bottles 9 and 10 to relieve the pressure.
9	Deactivate all valves.

Activator Plumbing Pressure Test

This test will check the 8, 11, and 4–port valve blocks, and the activator vessel and lines.

To test the activator plumbing pressure:

Step	Action
1	Activate valves 3, 11, 16, and 17.
2	Set the lower regulator to 11 psi.
3	Wait 1 min, and then turn the knob fully counterclockwise. **Note** Any pressure drop must be less than 0.30 psi in 3 min.
4	**IMPORTANT** Vent system pressure for the next test. Activate valve 1 to relieve pressure.
5	Deactivate all valves.

Reaction Vessel Plumbing Pressure Test

This test will check the reaction vessel, inline filters, conductivity cell, and the resin sampler valve.

Note This pressure test should be performed any time either reaction vessel inline filter is changed.

To test the reaction vessel plumbing pressure:

Step	Action
1	Place a metering vessel in the reaction vessel holder.
2	Activate valves 2, 10, 16, and 17.
3	Set the lower regulator to 11 psi.
4	Wait 1 min, and then turn the knob fully counterclockwise. You may see an initial pressure drop while backing out the regulators. **Note** Any pressure drop must be less than 0.30 psi in 3 min.
5	**IMPORTANT** Vent system pressure for the next test. Activate valve 1 to relieve pressure.
6	Deactivate all valves.

Needle Assembly Pressure Test

Perform the needle assembly pressure test whenever the needle assembly inline filter is changed. Prepare the needle assembly before performing the pressure test.

Needle Assembly

To prepare the needle assembly:

Step	Action
1	Check the Needle Down position. a. Using a knife or razor blade, cut open the side of an empty amino acid cartridge so that the bottom of the cartridge can be observed from the side. b. Remove the needle guard. c. Place the cut cartridge into the autosampler under the needle. d. Set the pusher block to hold the cut cartridge in place. The cut cartridge will act as your gauge. **Note** You don't need to use a cartridge; you can use a 0.05 in. Allen key instead to measure the needle down position.
2	Activate Function 5 (Needle Down). When the needle reaches the bottom, it should be very close to or barely touching the bottom of the cartridge. **IMPORTANT** The needle should not, under any circumstances, penetrate into the material of the bottom.
3	Activate Function 6 (Needle Up).
4	Insert an intact, empty cartridge with a new septum into the guideway.
5	Activate Function 5 (Needle Down).

Needle Pressure Test

To test the needle pressure:

Step	Action
1	Activate valves 12, 16, and 17.
2	Set the lower regulator to 11 psi.
3	Wait 1 min, and then turn the lower regulator knob fully counterclockwise.
4	Any pressure drop must be less than 3.0 psi in 2 min.
5	Activate valve 27 to relieve pressure.
6	Observe that pressure does, in fact, drop to near 0.
7	Deactivate all valves and raise the needle.

Resin Sampler Plumbing Pressure Test

To test the resin sampler plumbing pressure:

Step	Action
1	Install a plug at the resin sampler bulkhead fitting.
2	Activate function 88 (RS TO RV) and valves 8, 16, and 17.
3	Set the lower regulator to 11 psi.
4	Wait 1 min, and then turn the knob fully counterclockwise.
5	Any pressure drop must be less than 3.0 psi in 2 min.
6	Activate function 89 (RS TO FC) to relieve pressure.
7	Deactivate all valves.

Inspecting the System

Inspecting the Instrument

Inspect the instrument to determine that it is functioning properly.

To inspect the instrument:

Step	Action
1	Reset the instrument: a. With the instrument power OFF, remove the ROM cartridge. b. Turn instrument power ON for 5 to 10 seconds with the ROM cartridge removed. c. Turn instrument power OFF, and reinstall the ROM cartridge. d. Turn instrument power ON.
2	Verify the following conditions: a. The light behind the reaction vessel is on. b. The light behind the activator is on. c. The fan is on.
3	Look at the display. The display may indicate the chemistry of installation, *FastMoc*™ some other chemistry, or it may indicate: “Chemistry: NOT DECLARED!!” as in the example below. ABI 433A PEPTIDE SYNTHESIZER [Software Version] Chemistry: NOT DECLARED!! Main Menu ->

Running the Self-Test

To run the self-test:

Step	Action
1	Select **Main Menu**
2	Select **more**.
3	Select **self-test**.
4	Run **ALL** self-tests.

Calibration of the Barcode Reader

Calibrating the Barcode Reader

Any time the instrument is reset, the barcode reader must be calibrated.

To calibrate the barcode reader using the Barcode Calibration Menu:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Select more from the main menu.</td></tr>
<tr><td>2</td><td>Select barcode reader.</td></tr>
<tr><td>3</td><td>Select calib.</td></tr>
<tr><td>4</td><td>Put the calibrating cartridge (PN 400269) in the guideway.</td></tr>
<tr><td>5</td><td>Place the pusher against the cartridge.</td></tr>
<tr><td>6</td><td>Lower the retaining bar.</td></tr>
<tr><td>7</td><td>Press enter.
The following messages will appear:
♦ Reading barcode.
♦ Turn calibrator cartridge around.</td></tr>
<tr><td>8</td><td>Install the cartridge with the opposite set of bands to the Barcode Read head.</td></tr>
<tr><td>9</td><td>Press enter.
One of two messages will appear.
<table>
<tr><th>If the message is...</th><th>Then...</th></tr>
<tr><td>“Barcode Reader Is Calibrated”</td><td>Your channel values are 2000 ± 500.</td></tr>
<tr><td>“Barcode Reader Needs Service”</td><td>♦ Your channel values are
– <1000
– >2500
♦ Repeat the calibration several times noting the values seen.
♦ If the values continue to fall outside of specifications, do the following:
– Align the barcode reader, if needed.
– Adjust the potentiometers on the distribution board, if needed.
– Refer to your Service Manual.</td></tr>
</table></td></tr>
<tr><td>10</td><td>Record the channel gains on the installation report.</td></tr>
</table>

SynthAssist®3.1Software 4

Overview

About This Chapter

This chapter describes SynthAssist® 3.1 Software, a Peptide Synthesis software program that runs on a PC and communicates with the ABI 433A Peptide Synthesizer. Included in the chapter are procedures for creating user chemistry files.

In This Chapter

This chapter covers the following topics:

Topic	See Page
About SynthAssist® Software	4-2
Installing SynthAssist® 3.1 Software	4-2
Creating User Chemistry Files	4-2

About SynthAssist® Software

For complete information about SynthAssist® Software, refer to Chapter 2, Introduction, in the *SynthAssist® Software Version 3.1 User Guide* (PN 4338849, Rev. B).

Installing SynthAssist® 3.1 Software

Operating System Requirements

IMPORTANT Operating System Windows 2000 (SP4) or XP Professional (SP1 or higher) must be installed to run the Software disc.

Installing SynthAssist® on the Hard Drive

Before you install the software, you need to connect the API 433A Peptide Synthesizer to the computer.

Refer to Chapter 2, Introduction, in the *SynthAssist® Software Version 3.1 User Guide* (PN 4338849, Rev. B) for complete connection and installation instructions.

Note The comct32.ocx file must be present in the system32 folder.

Establishing Communication with the 433A Peptide Synthesizer

Before you can send chemistry or run files to the synthesizer, you need to establish communications between the PC and the 433A Peptide Synthesizer.

Refer to Chapter 4, Communications, in the *SynthAssist® Software Version 3.1 User Guide* (PN 4338849, Rev. B) for instructions.

Creating User Chemistry Files

Creating a User Chemistry Folder

The SynthAssist® software includes pre-defined Chemistry files. By default, these files are locked. You need to create a User Chemistry folder, and then save and unlock copies of the desired Chemistry files in the User Chemistry folder.

To create a folder:

Step	Action
1	In My Computer, navigate to the C:\Program Files\Applied Biosystems\SynthAssist3.1 folder.
2	Select **File >New > Folder.**
3	Name the new folder “User Chemistry”.

Copying and Unlocking the Chemistry Files

To copy and unlock the Chemistry files:

Step	Action
1	Click **File > Open** from the SynthAssist® Software main menu. The File Type dialog box opens.
2	Click **Chemistry** to open the main Chemistry folder.
3	Navigate to the desired Chemistry file.
4	Double-click the file name to open it.
5	Click **File > Save As...**
6	In the Save As dialog box, navigate to the User Chemistry folder.
7	Type a name for the chemistry file, and click **Save**.
8	When the Lock Chemistry file? message appears, click **No**.
9	Repeat steps 1 through 8 for all chemistry files appropriate to your customer's application.

Flow Tests 5

Overview

About This Chapter

This chapter presents general information about flow tests. Details on running flow tests are described in Appendix A.

Precautions for loading chemicals on the instrument and an itemized list of the contents of the *FastMoc*™ kit are presented. Directions for selecting and downloading flow tests are described. Directions for setting the gas regulators and performing flow tests for *FastMoc*™ reagent installation are presented, also.

In This Chapter

This chapter covers the following topics:

Topic	See Page
Downloading Flow Tests 1–18	5-2
Installing FastMoc™ Chemicals	5-4
Selecting and Running a Flow Test	5-7
About Flow Testing	5-9
Setting Regulators	5-10
Performing Flow Testing for FastMoc™ Reagent Installation	5-12
Before Proceeding to FastMoc™ Chemistry Installation	5-13

Downloading Flow Tests 1–18

Establishing Communication with the Synthesizer

Using the computer, download the flow tests in the same manner as you would when changing chemistries.

To establish communication with the synthesizer:

Step	Action
1	Go to the **Synthesizer** pull-down menu, and choose **Connect**. A dialog box will appear.
2	Click **Communications enabled**, select **COM1** port, and click **OK**.

Synchronizing the Time on the Instrument and the Computer

The time on the computer and the ABI 433A instrument should be synchronized.

To synchronize the time:

Step	Action
1	Go to the **Synthesizer** pull-down menu.
2	Select **Set Clock**.
3	Click **Set** in the dialog box if the times or dates are not synchronized. **Note** The time and date are automatically synchronized when you click **Set**.
4	Click **OK** in the dialog box. **Note** In some cases, it may be necessary to reboot the instrument to reestablish communication.

Selecting Flow Tests 1–18

To download Flow Test 1–18:

Step	Action
1	Open the User Chemistry folder.
2	Find the file **Flow Tests 1–18** within the User Chemistry folder.
3	Double-click on **Flow Tests 1–18** to open the file.

Downloading Flow Tests 1–18

Download the flow tests using the following procedure.

To download the flow tests and run all self-tests:

Step	Action
1	Select the **Synthesizer** option on the menu bar.
2	Select **Send.**
3	A dialog box opens. ♦ The chemistry for which the synthesizer is currently set appears at the top (*e.g.*, NOT DECLARED). ♦ The name of the chemistry to be sent appears at the bottom (in this case, **Flow Tests**).
4	Press **RETURN** or click the **SEND** box. The modules will begin to download, and the ABI 433A instrument will “chirp” as each module is received.
5	When all modules have been transmitted and the dialog box clears, press the **MAIN MENU** key twice on the ABI 433A instrument. The display should read as follows: ABI 433A PEPTIDE SYNTHESIZER [SoftwareVersion] Chemistry: Flow Tests 1-18 Main Menu ->

Installing *FastMoc*™ Chemicals

Precautions

⚠ WARNING **CHEMICAL HAZARD**. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or special clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (*e.g.,* fume hood). For additional safety guidelines, consult the MSDS.
- Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.

Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

⚠ WARNING **CHEMICAL HAZARD**. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

⚠ WARNING **CHEMICAL HAZARD**. Ensure all fittings are properly connected, all leak tests have been successfully passed and the instrument venting is properly installed before loading chemistry on the instrument.

IMPORTANT Use the appropriate seals when loading synthesizing chemicals on the instrument.

Loading Chemicals for Flow Tests

IMPORTANT Verify that piperidine is present onsite before the installation date. Piperidine is a controlled substance. This must be ordered by the customer, and it is not provided in the chemical installation kit. See “Ordering Piperidine” on page 2-2 for more information.

To load chemicals for flow testing, install the external bottle caps with the parallel bottle cap assemblies at positions 9 and 10. Pay careful attention to the P and D markings, which indicate the lines that must be attached to the pressure and delivery ports, respectively.

Load the chemicals in the bottles as indicated in the following table:

Bottle Contents for Flow Tests:

Bottle No.	Contents	Notes and Instructions
1	Piperidine	**Note** See “Bottles with Polyethylene Seals” below.
2	None	
4	None	
5	0.45 M HBTU/HOBt/DMF	Pour contents of 200-mL bottle of HOBt/DMF (PN 400934) into the 450-mL bottle containing the HBTU powder (PN 401091), and mix according to directions supplied with the kit. Be sure to install a filter on the line as directed in those instructions. **IMPORTANT** The label on the bottle of *FastMoc*™ reagent HOBt/DMF is marked with a (**7**) to indicate bottle position, but the bottle DOES NOT go on the instrument at all. This bottle is part of the HBTU kit and must be mixed according to directions that come with the kit. **Note** See “Bottles with Polyethylene Seals” below.
6	None	
7	neat NMP	Install neat NMP, filling the bottle 1/4 full. After the initial flow tests, you are prompted to install the standard chemicals for bottle 7, 2.0 M DIEA/NMP. **Note** This bottle label has no “bottle number” on it, because it is used in different positions on different synthesizers. **Note** See “Bottles with Polyethylene Seals” below.
8	neat NMP	Install neat NMP, filling the bottle 1/4 full.
9	DCM	
10	NMP (2 bottles)	

Bottles with Polyethylene Seals

The bottles are installed with polyethylene seals as indicated in the following table.

FastMoc™ reagent bottles requiring polyethylene seals:

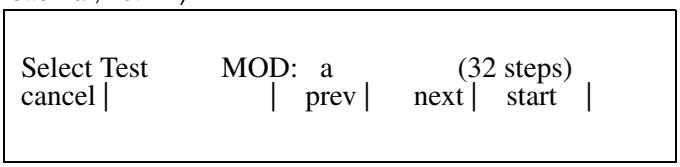
Bottle No.	Bottle Size (oz.)	Part Number
7	8	400790 (seal)
1 and 5	16	400501 (seal)

IMPORTANT The seals for bottles should never be reused.

Selecting and Running a Flow Test

Selecting a Flow Test

To select a particular flow test from a group of downloaded flow tests:

Step	Action
1	Go to the Main Menu of the instrument and choose **module test**.
2	Press **next** until the display shows the desired module (see chart below). For example, for Flow Test 1, press **next** until the display shows **MOD: a** (small letter “a”, not “A”). 
3	Verify that either the metering vessel or cartridge is installed for the appropriate flow test.
4	Select **Start** to begin the flow test procedure.

List of Flow Tests and Modules

Module	Flow Test	Definition
File: Flow Tests	1–18	—
a	1	Bottle 1 to RV
b	2	Bottle 2 to RV
c	3	Barcode--> Printer
d	4	Bottle 4 to RV
e	5	Bottle 5 to RV
f	6	Bottle 6 to RV
g	7	Bottle 7 to Waste
h	8	Bottle 8 to Waste
i	9	Bottle 9 to RV
A	10	Bottle 10 to RV
B	11	Bottle 10 to Cartridge
C	12	Bottle 9 to Cartridge
D	13	Bottle 5 to Cartridge
E	14	Bottle 10 to Act --> RV --> Cart
F	15	Bottle 9 to Act Top with Drain
G	16	Bottle 10 to RV Top
H	17	Measure 7 to Cartridge
I	18	Measure 8 to Cartridge

Running a Flow Test

For information about how to run a particular flow test, see Appendix A, which lists each flow test's steps, standards for deliveries, and further information on how to perform a particular test.

About Flow Testing

Overview

IMPORTANT Flow tests must be performed prior to the *FastMoc*™ chemistry synthesis.

The software includes a series of flow tests to check the operation and calibration of the instrument. Read “Downloading Flow Tests 1–18” on page 5-2 for information on how to download flow tests and “Selecting and Running a Flow Test” on page 5-7 for how to use them. Further details of these tests including the standards of performance are included in Appendix A. See this appendix for current flow test information and proper specifications.

General Directions for Flow Testing

General directions for running flow tests.

To run flow tests:

Step	Action
1	Place an empty cartridge in the autosampler before starting a flow test.
2	Run each flow test at least twice to confirm accuracy. **Note** The first reading from a flow test should be discarded and not used in calculations. Therefore, each test must be performed a minimum of three times.
3	Record each measurement on the Installation Report.

Setting Regulators

Setting the Lower Regulator with Flow Test 10

IMPORTANT The regulators that control liquid delivery must be correctly set for the ABI 433A instrument to perform properly.

The following procedures require performance of certain flow tests to achieve proper regulator settings, and further flow tests will be performed to verify the integrity and proper assembly of the plumbing system.

This regulator controls delivery of all reagents other than Bottle 2 (TFA for Boc applications); gas pressure to the eight-port valve block, from which the other blocks are pressurized; and delivery of gas pressure to the input of the upper regulator, which is used for Bottle 2 (TFA) delivery.

To calibrate the lower regulator:

Step	Action
1	Set the regulator for the pressure shown on the Final Test documentation. **Note** If a final test pressure is not available, set the regulator to 9.5 psi.
2	Toggle pressure/vent switches 9 and 10 to the pressurize (UP) position.
3	If Flow Test 1–18 is not on the instrument, follow the instructions for “Establishing Communication with the Synthesizer” on page 5-2 from the ABI Chemistries Folder. **Note** Read about the use of flow tests under the heading “Selecting and Running a Flow Test” on page 5-7. Refer to Appendix A for specific Flow Test information.
4	Install the Metering Vessel in the RV Holder.
5	Select Flow Test 10 (**Mod: A**). ♦ This test delivers the contents of Bottle 10 to the reaction vessel position. ♦ With NMP in Bottle 10, 2.5 mL ±0.10 mL should be delivered to the metering vessel.
6	Confirm regulator setting with Flow Test 11 (Module B). ♦ This test delivers the contents of Bottle 10 to the cartridge. ♦ With NMP in Bottle 10, 1.95 to 2.35 g should be delivered to the cartridge.

Increasing the Lower Regulator Range with Flow Test 11

Sometimes you will discover that Flow Test 11 may fail in later testing. In that event, the lower regulator can be readjusted to provide up to 2.75 mL in Flow Test 10, which will often allow Flow Test 11 to come into specification.

Note Use this larger range only if you have first cleared any problems from the instrument, installed clean filters and tried and failed Flow Test 11.

IMPORTANT The lower regulator should never be set for a value higher than 11 psi. If a higher value is required to obtain correct delivery, it indicates a delivery problem in the instrument.

If...	Then...
less than the required volume is delivered	troubleshoot as follows: a. Turn the lower regulator clockwise 1/8 to 1/4 turn, and wait at least 60 seconds for the pressure to stabilize. b. Repeat the flow test. Continue readjusting the regulator if necessary, and run the flow test until the correct volume is achieved. c. Confirm regulator setting with Flow Test 11 (Mod: B), NMP to cartridge. d. Repeat the flow test until two consecutive deliveries fall within specification.
if more than the desired volume is delivered	troubleshoot as follows: a. Switch the vent valve for bottles 9 and 10 to the “vent” position. b. Turn the lower regulator counterclockwise 1/8 to 1/4 turn. c. Return the manual valve to the “pressure” position. d. Wait at least 60 seconds for the pressure to stabilize. e. Repeat the flow test. f. Continue readjusting the regulator if necessary. g. Rerun the flow test until the desired volume is achieved. h. Repeat the flow test until two consecutive deliveries fall within specification.

Confirm the calibration of the metering loop. Follow the procedure in Appendix B, “Metering Loop,” of this guide.

Performing Flow Testing for *FastMoc*™ Reagent Installation

Perform Flow Testing

To begin, fill bottles 7 and 8 approximately 1/4 full with NMP.

For each of the following flow tests, repeat the test until 3 consecutive readings are within the appropriate range for each test.

Perform flow testing in the following order:

Step	Action
1	Run flow test 17 to measure bottle 7 to the cartridge. Expected result: NMP, 0.48 to 0.554 g. Then, replace the neat NMP in bottle 7 with the standard DIEA/NMP solution. If unable to get results in the range, see Appendix B, “Metering Loop,” to learn how to calibrate the metering loop.
2	Then run flow test 7 three times to flush the remaining neat NMP from the flow path. Also, watch the metering loop and verify that it fills within 2 to 3 seconds. No measurements are taken.
3	Run flow test 10 to deliver NMP to the Reaction Vessel. Expected result: NMP, 2.4 to 2.6 mL.
4	Run flow test 11 to deliver NMP to the cartridge. Expected result: NMP, 1.95 to 2.35 g.
5	Run flow test 17 to deliver DIEA to the cartridge. Expected result: DIEA, 0.42 to 0.50 g
6	Run flow test 6 to deliver MeOH to the Reaction Vessel. Expected result: MeOH, 1.95 to 2.55 mL.
7	Run flow test 1 to deliver piperidine to the bottom of the Metering Vessel. Expected result: piperidine, 0.80 to 1.30 mL
8	Run flow test 9 to deliver DCM to the bottom of the Metering Vessel. Expected result: DCM, 2.8 to 3.5 mL
9	Run flow test 12 to deliver DCM to the needle line. Expected result: DCM 3.4 to 4.10 g
10	Repeat flow test 11 twice to remove excess DCM from the needle line and prevent precipitation of the HBTU. No measurements are taken.
11	Run flow test 13 to deliver HBTU to the needle line. Expected result: HBTU, 1.9 to 2.1 g
12	Repeat flow test twice 11 to remove excess HBTU from the needle line. No measurements are taken.
13	Run flow test 14. Flow Test 14 is a two-part flow test: a. A delivery of NMP (Bottle 10) is made to the activator (ACT), and then transferred to the metering vessel. b. A delivery of NMP is made to the cartridge, transferred to the ACT, and finally to the metering vessel. Expected result, step #10: NMP, 2.0 to 2.8 mL step #28: Minimum of 1mL NMP

Perform flow testing in the following order: *(continued)*

Step	Action
14	Run flow test 16. A delivery of NMP is made to the top of the metering vessel. Expected Result: NMP, 0.90 to 1.30 mL
15	Run flow test 8. This test is done to confirm that no leaks are in the delivery line from bottle 8. No measurements are taken.

Before Proceeding to *FastMoc*™ Chemistry Installation

You will use only one of the following chapters to complete the next step, *FastMoc*™ Installation and Performance Run:

- Chapter 6, “FastMoc™ Chemistry/ Conductivity Installation & Performance Run,” if the customer **has not purchased** the UV Monitoring Accessory Kit.
- Chapter 7, “FastMoc™ Chemistry/UV Installation & Performance Run,” if the customer **has purchased** the UV Monitoring Accessory Kit.

FastMoc™ Chemistry/ Conductivity Installation & Performance Run

6

Overview

About This Chapter

This chapter provides information for evaluating instrument performance using a di-mer peptide. Synthesis is performed only after the startup procedure is complete and the flow test specifications are met.

Directions for performing presynthesis checks, preparing the resin, performing the run, and evaluating the run in terms of the deprotection peaks are included. Criteria for determining if the installation is complete are listed at the end of the chapter.

Note The conductivity cell used on the Applied Biosystems 433A Peptide Synthesizer monitors Fmoc removal in real time by measuring a carbamate salt that is generated during the piperidine deprotection. This online feedback monitoring provides the capacity to automatically perform additional deblocking steps within the synthesis run, resulting in improved overall yield. However, the conductivity cell also detects salts from other chemicals and solvents that can produce misleading conductivity data. An alternative to monitoring the deprotection chemistry is to monitor the production of the fulvene-piperidine adduct by spectrophotometric detection. If the customer has purchased the UV Monitoring Accessory Kit, then skip this chapter and go to Chapter 7, "FastMoc™ Chemistry/UV Installation & Performance Run," for complete information and instructions.

In This Chapter

This chapter covers the following topics:

Topic	See Page
Di-Peptide Synthesis	6-2
Presynthesis Check	6-2
Creating the Di-Mer Sequence and Run	6-4
Preparing the Resin	6-5
Performing and Evaluating the Run	6-7
Completing the FastMoc™ Chemistry Installation	6-10

Di-Peptide Synthesis

About

Once the entire startup procedure is complete and all the flow test specifications are met, synthesis of a standard test peptide is necessary to evaluate instrument performance.

Note The instrument performance functionality will be verified, according to the manufacturer's specifications, by leak tests, chemistry specific flowtests, and the running of a Di-mer (Phe-Gly) on *FastMoc*™ chemistry with conductivity monitoring or *FastMoc*™ chemistry with UV monitoring.

Certain chemicals used in the testing are not supplied by Applied Biosystems but by independent third party vendors therefore results from Conductivity or UV Monitoring may be affected by the quality of the chemicals used in the testing and such effects will be reflected in the background height and number of deprotection peaks obtained for each Amino Acid. The variability in the background height and/or number of Deprotection Peaks is not necessarily an indication of the instrument not performing according to the manufacturer's specifications.

Synthesis to Evaluate Instrument Performance

The results must meet or exceed the minimum installation performance, based on evaluation of the monitoring trace, for the installation to be considered satisfactory. All flow tests previously specified must meet specification prior to performing this synthesis.

As the synthesis begins, check for plumbing leaks, and ensure that the sides of the activator vessel are "sheeting" well when rinsed. (All areas of the vessel should get rinsed by liquid). Watch at least the entire first cycle of the synthesis (first amino acid addition) to observe activation and transfer to the reaction vessel.

Note Because the chemistry kit includes a preloaded resin (Gly), only one coupling cycle is required.

Presynthesis Check

Presynthesis Instrument, Reagent and Application Check

Before beginning the synthesis of a test peptide, it is necessary to check the equipment, reagents and application.

Step	Action
1	Perform a presynthesis instrument check as follows: a. Check that liquid levels of reagents and solvents are adequate for synthesis. b. Check that the waste container is empty.
2	Obtain: ♦ Necessary amino acid cartridge ♦ A vial of resin ♦ A small-scale (8-mL) reaction vessel

Verifying Functions in the Deprotection and Activation Modules

Functions in the Deprotection module must be verified and step 13 in the Activation module must be edited before beginning the synthesis.

To verify functions in the deprotection and activation modules:

Step	Action
1	Select **File > Open**.
2	Choose ***FastMoc***™ **0.1Ω MonPrev Pk**, contained in the USER Chemistry Folder.
3	Open **Module B (Deprotection/Ω MonPrev Peak)** under the Modules column in the chemistry file.
4	Verify at step 33, FXN 133 (Begin loop monitoring), that the value **3** appears in the time field.
5	Verify at step 61, FXN 134 (End loop monitoring), that the value **100** appears in the time field. **Note** This default value should not be changed prior to the install run.
6	Click the upper-left corner box to close the B module.
7	Open **Module A (Activation)** under the Modules column. a. Scroll down to step 13, FXN 94 (Deliver HBTU to Cartridge). b. Enter the appropriate **5 to Cartridge time** from Flow Test 13. c. Press the **ENTER** key after modifying the time for FXN 94; otherwise, changes will not be saved.
8	Close Module A by clicking on the upper-left corner box of the window.
9	Save the chemistry changes by selecting **Save** from the **File** pull-down menu.
10	Send the ***FastMoc***™ **0.1Ω MonPrev Peak** chemistry to the ABI 433A instrument. a. Select **Synthesizer** and **Send** from the Menu Bar. A series of chirps will be audible from the ABI 433A instrument when modules arrive to the instrument (assuming, communication is enabled). b. The display should read as follows: ABI 433A PEPTIDE SYNTHESIZER [Software Version] Chemistry: *FastMoc* 0.10 Mon Prev Pk Main Menu ->

Creating the Di-Mer Sequence and Run

Creating the Di-Mer Sequence

Step	Action
1	Select **File > New**. A dialog box opens.
2	Select **Sequence**.
3	Enter the sequence **Phe - Gly**.
4	Save as **Di-mer test**.

Creating a Run for the Synthesis

Step	Action
1	Select **File > New**. A dialog box opens.
2	Select **Run** for File Type. An untitled run file opens on the computer screen.
3	Select ***FastMoc***™ **0.1Ω MonPrev Peak chemistry** (previously edited) from the top pop-up window of the run page.
4	From the second pop-up window, create sequence from the Sequence folder.
5	On the Calculations page, click **Amide resin** in the upper-left corner.
6	In the pop-up window, select **Preloaded** , then press the **return** key to save the resin changes in the Calculations window.

Preparing the Resin

Calculating the Amount of Resin Needed for Synthesis

Step	Action
1	Obtain the loading or substitution value in mmol/gram from the resin vial label.
2	Calculate the required weight of resin: a. Enter the substitution value for the resin in the appropriate column. b. Press the **Enter** key to save the change. c. Enter **0.1** in the Resin mmol column. – The calculation sheet automatically calculates the amount of resin you need for synthesis at this 0.1-mmol scale and displays it under “weight g”. – You can also calculate the amount of resin by the formula: 0.1 mmol/substitution value (mmol/g) – Typical substitution values range from 0.6 to 0.9 mmol/gm. For example, a substitution of 0.72 mmol/g would require: 0.1 mmol/ 0.72 mmol/g= 0.139 g or 139 mg of resin
3	**SAVE** the run in the User run folder; it is saved as Run ##.

Sending the Run File to the ABI 433A Synthesizer

To send the run file to the ABI 433A synthesizer:

Step	Action
1	Select **Synthesizer > Send**. ♦ A chirp sounds when the ABI 433A instrument receives the run file. ♦ A log file is generated automatically when a run file is sent to the instrument.
2	Open the monitor window of the run file to view the deprotection peaks.
3	Save the log file to a log folder: a. Select **File > SAVE AS**. b. Enter a run name or date in the dialog box.
4	Select **File > Auto-SAVE**. The Auto-SAVE featuresaves collected data every 5 min. ♦ If power is lost and restored, the Auto-SAVE feature ensures that the last 5 minutes of collected data before the power outage is available. ♦ The default time is 5 minutes, but this value can be changed to less than 5 minutes.

Weighing the Resin

Weigh the required amount of resin into the small-scale reaction vessel (RV).

Step	Action
1	Place a tabbed RV filter (PN 401524) in the opening of the vessel, seating it on the interior ledge of the small scale reaction vessel body: a. Screw the bottom cap over the RV opening with the filter, holding the RV in a vertical position at all times. b. Tighten the cap until the first resistance is felt, wait a few minutes, then resume tightening. **IMPORTANT** DO NOT overtighten the caps to the RV body because the threads will crack.
2	Invert the RV to add the resin: a. Weigh out the required amount of resin into the RV. See "Calculating the Amount of Resin Needed for Synthesis" on page 6-5. b. Install the tabbed filter at the upper ledge of the RV. c. Install the top RV cap assembly. **Note** Each preloaded glycine vial contains enough resin for two syntheses plus some excess.
3	Record the lot number of the preloaded resin, loading, and actual resin weight on the Run Sheet of the Installation Report.

Placing the Reaction Vessel in Its Holder and Installing the Amino Acid

Step	Action
1	Place the assembled RV containing the resin in its holder.
2	Slide the pusher all the way to the left and lock it in place.
3	Remove the foil cap insert to expose the septum on the amino acid cartridge.
4	Record the lot number of the cartridge on the Run Sheet.
5	Place an *empty* cartridge in the guide rail, followed by the Phe amino acid cartridge. **Note** The first amino acid, Glycine, is already on the resin.
6	Verify the loading order in the SynthAssist® application by selecting **Cartridges** in the Calculations pop-up window of the Run file.
7	Slowly release the pusher and the cartridge retention bar.

Performing and Evaluating the Run

Starting the Run

Step	Action
1	Return to the Main Menu on the instrument front panel.
2	Select the following in sequence: a. **cycle monitor** b. **Resin Sampling?** – Answer **NO** – This is the default condition and you will normally only need to press **CONTINUE** c. **Hold Times** – Answer **0 (zero)** – This is the default condition and you will normally only need to press **CONTINUE** d. **...print events?** Select **no**. e. **begin**
3	Check for leaks from the RV.
4	View the first set of deprotection peaks to verify that they are of normal height similar to those in Figure 6-1 on page 6-9.
5	After the run is complete, have the customer save the run and the log file. **Note** The run completes in approximately 1 to 1.5 hours.

Passing Criteria for the Run

Passing criteria for the run are based on the evaluation of the monitoring data. An example is shown in Figure 6-1 on page 6-9. The criteria are based on the number of deprotection peaks observed for each residue of the peptide.

Using Table 6-1, the example in Figure 6-1 meets the passing criteria because the number of deprotection peaks observed are within the range of those expected.

Table 6-1 Expected number of deprotection peaks for each residue for conductivity installations

Cycle	Residue	Minimum Number of Deprotection Peaks for Each Residue
1	Gly (Preloaded)	3
2	Phe	3

Examples of Deprotection Peak Criteria

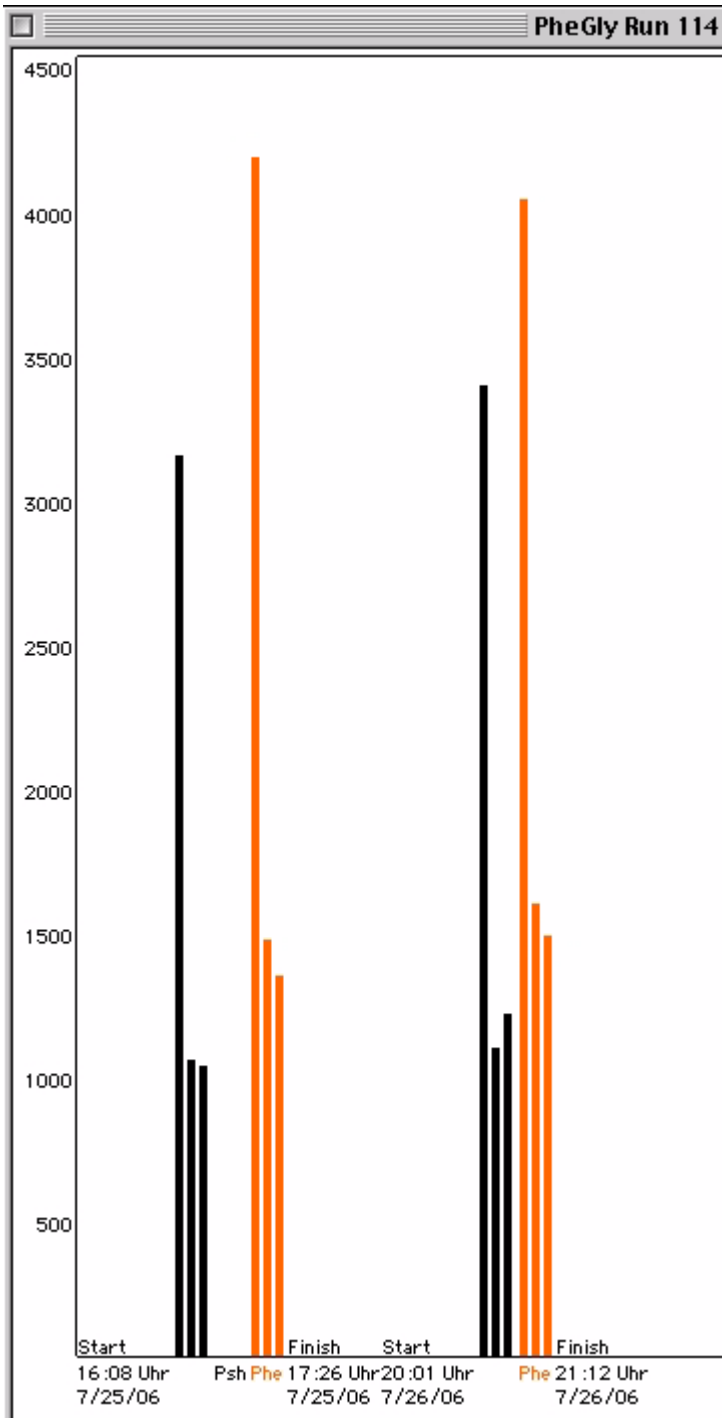


Figure 6-1 Example of deprotection peak criteria for determining a successful run

Completing the *FastMoc*™ Chemistry Installation

Complete Installation

FastMoc™ chemistry installation is complete if:

- The data values and all flow test results meet or exceed the minimum values required.
- Customer training is complete, that is, the customer has been trained in:
  - Theory
  - Operation
  - Maintenance
- The warranty policy has been explained to the customer.
- All paperwork is complete and the customer has signed the:
  - Service Order
  - Installation Report

Have the customer discard both the finished product from the RV and the used RV filters in accordance with information in the MSDS and waste profile.

FastMoc™ Chemistry/UV Installation & Performance Run 7

Overview

About This Chapter

Note If the customer has purchased the UV Monitoring Accessory Kit, then use this chapter instead of the instructions in Chapter 6, "FastMoc™ Chemistry/ Conductivity Installation & Performance Run."

This chapter provides information for evaluating instrument performance using a di-mer peptide. Synthesis is performed only after the startup procedure is complete and the flow test specifications are met. This chapter provides instructions for an alternative to monitoring the deprotection chemistry described in Chapter 6. UV monitoring provides improved detection sensitivity and avoids problems associated with salts and other conductive species. The UV Monitoring Accessory Kit has been configured to connect with the 433A Peptide Synthesizer to carry out deprotection monitoring as a replacement for the standard conductivity cell. The UV Monitoring Accessory Kit provides increased sensitivity and full feedback monitoring of the deprotection chemistry. This chapter explains how to install the UV Monitoring Accessory Kit, how to configure, calibrate, and test the monitoring system, and how to perform and evaluate the tests. Directions for performing presynthesis checks, preparing the resin, performing the run, and evaluating the run in terms of the deprotection peaks are included.

In This Chapter

This chapter covers the following topics:

Topic	See Page
Pre-installation Check	7-2
Assembling the Detector Output Cable	7-3
Installing the Detector	7-3
Installing the Flowcell	7-5
Configuring the Detector	7-10
Calibrating the Monitoring System	7-12
Di-Peptide Synthesis	7-14
Presynthesis Check	7-14
Creating the Di-Mer Sequence and Run	7-15
Preparing the Resin	7-16
Performing and Evaluating the Run	7-18
Completing the FastMoc™ Chemistry Installation	7-23

Pre-installation Check

Verifying the Receipt of Materials

If your customer purchased the UV Monitoring Accessory Kit with this installation, verify that the the following parts have been received:

Part Number	Part Description and Components	
4335867	**Kit, UV Monitoring Accessory**	
	Part Number	
	4322246	Series 200 UV/Vis detector (S200) *
	2900-0542	Standard analytical flowcell, 8 mm × 12 µL
	2900-0152	Microbore head adaptor
	4336090	**Kit, 433 UV Chemistry for S200**
	4335681	433A UV cycles on CD
	4336048	433A UV cycles on diskette
	4332159	**TBG Assy Kit**
	4332156	Assy, TBG PTFE Yellow 28.0" LG
	4332157	Assy, TBG PTFE Red 45.0" LG
	4332158	Assy, TBG PTFE Blue 35.0" LG
	4335868	UV Detection Tubing Spares Kit
4335884	**UV Chemical Install Kit**	
	Part Number	**Part**
	400847	Fmoc-Gly resin, 0.25 mmol
	400645	Fmoc-L-Phenylalanine
	400470	Methanol

*. The Series 200 UV/Vis detector is shipped with the *PerkinElmer Series 200 UV/Vis Detector User Manual* (Rev A). This chapter often refers to the PerkinElmer guide for more information. Please ensure the guide is included in the box.

Assembling the Detector Output Cable

To assemble the output cable for the S200 detector:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Locate one of the two 3-port (green) terminal strips (PE PN 999-7562) and the signal cable (PE PN N235-0157). Both items are in the S200 accessory kit.

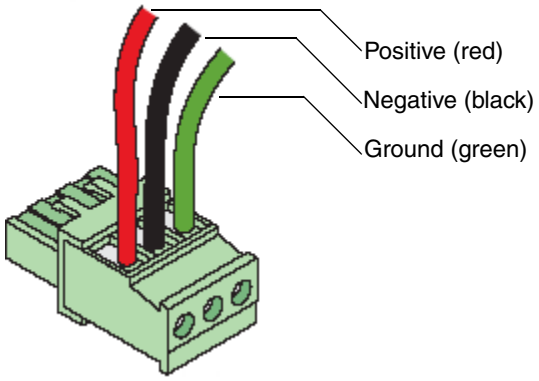
IMPORTANT The signal cable has two distinct ends:

♦ One end has a positive and a negative wire (2-wire end).
♦ The other end has a positive, a negative, and a green ground wire (3-wire end).

Note Wires should be labeled when you receive them.</td></tr>
<tr><td>2</td><td>Connect the wires from the 3-wire end of the signal cable to the ports on the 3-port terminal as follows:

<table>
<tr><th>Color</th><th>Port Position</th><th>Symbol on the Instrument</th></tr>
<tr><td>Red</td><td>Left</td><td>+ (positive)</td></tr>
<tr><td>Black</td><td>Center</td><td>– (negative)</td></tr>
<tr><td>Green</td><td>Right</td><td>⏚ (ground)</td></tr>
</table>

Note The 3-port terminal strip is connected to the instrument such that the wires are on top (pointing up). When the terminal strip is properly connected to the instrument, the wires are from front to back: red, black, and green.



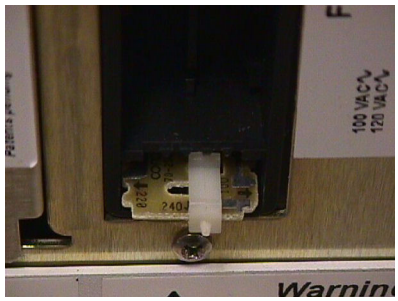
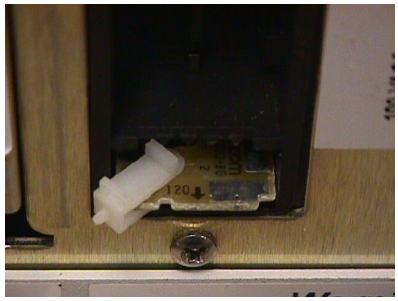
</td></tr>
</table>

Installing the Detector

To install the Series 200 UV/Vis detector:

Step	Action
1	Install the two 1.6 A fuses, found in the accessories box in the detector crate (PN 5100-0162)

To install the Series 200 UV/Vis detector: *(continued)*

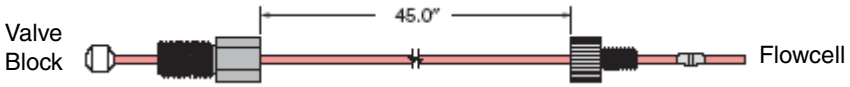
Step	Action
2	At the rear of the detector, turn the voltage selector card to the appropriate voltage so that the desired voltage is marked on the inward end. See examples below, or refer to pages 2-6 in the *PerkinElmer Series 200 UV/Vis Detector User Manual* (Rev. A). The white pin protrudes through the fuse drawer for the corresponding voltage setting.  Voltage card in 120 V configuration  Voltage card in 240 V configuration

Installing the Flowcell

To install the flowcell:

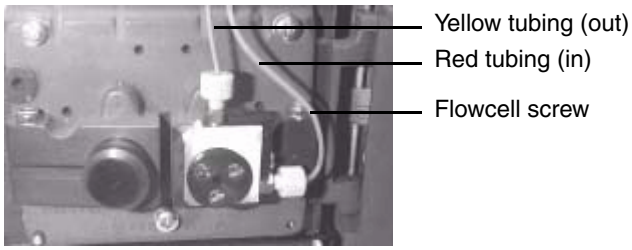
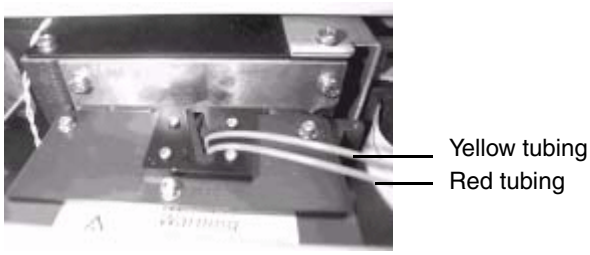
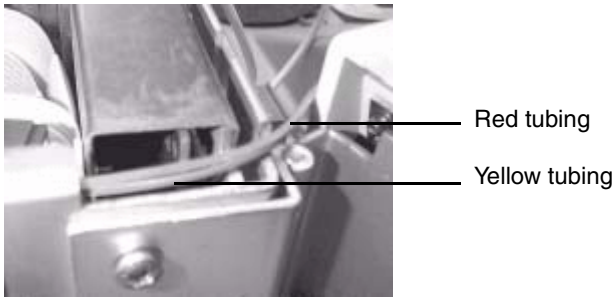
Step	Action
1	Open the flowcell compartment: a. Loosen the knurled nut on the upper-right corner of the back panel. b. Open the access door. c. Unscrew the knurled nut that is holding the front panel. d. Swing open the front panel to the right. e. Slide the drawer out to the first stop. Be careful that the drawer is supported and does not tip.
2	Locate the microbore head adapter (PN 2900-0152) in the UV kit. Remove the standard plate on the S200 and replace it with the microbore head adapter. Refer to “Attaching the Flowcell” step 2 on page 8 of the *PerkinElmer Series 200 UV/Vis Detector User Manual* (Rev A) for a picture of the microbore head adapter and ferrules.
3	Locate the flowcell (PN 2900-0542) and remove the two stainless steel nuts and ferrules.

To install the flowcell: *(continued)*

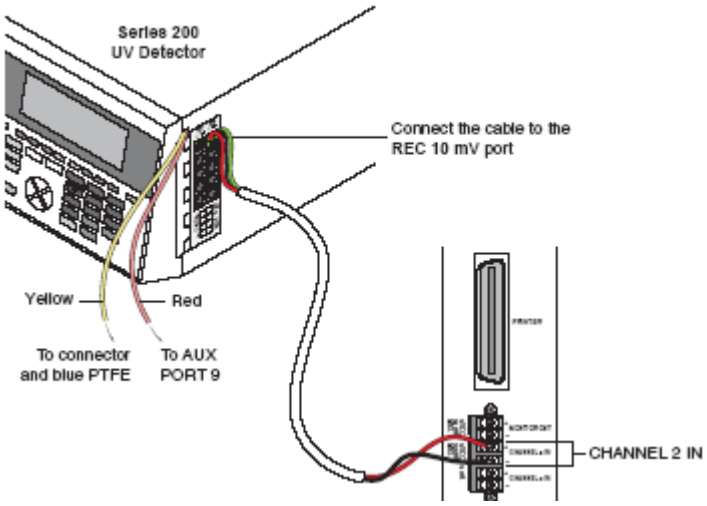
Step	Action
4	Locate the three tubes in the UV kit. Blue PTFE (PN 4332158) Red PTFE (PN 4332157)  Yellow PFTE (PN 4332156)
5	Connect the end of the red PTFE tube (PN 4332157) to the flowcell IN position. Gently insert the end of the tube with the double-sided ferrule until the tube is touching the back of the housing, then fingertighten.
6	Connect the end of the yellow PTFE tube (PN 4332156) to the OUT position of the flowcell in a similar manner to step 5.
7	Open the flowcell door. **Note** Refer to Figure 7-1 on page 7-5 of the *PerkinElmer Series 200 UV/Vis Detector User Manual* (Rev A) for a picture showing the location of the flowcell door.

Attaching the Flowcell

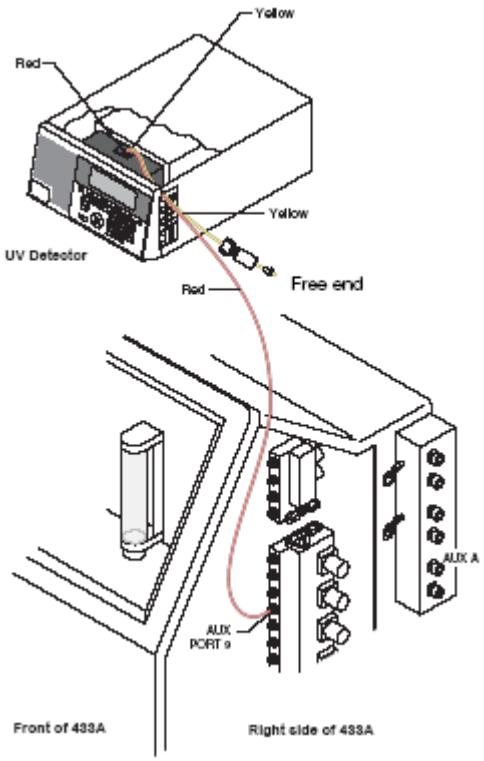
To attach the flowcell:

Step	Action
1	Attach the flowcell to the detector. Before tightening the flowcell, make sure it is straight. Hold the flowcell with one hand while tightening the flowcell screw with the other hand. **IMPORTANT** Do not overtighten the flowcell fitting; damage to the flowcell can result. **IMPORTANT** Position the flowcell so that the exit port is vertical. **Note** Refer to Figure 7-1 on page 7-5 of the *PerkinElmer Series 200 UV/Vis Detector User Manual* (Rev A) for a picture showing the location of the flowcell screw. The picture in Figure 7-1 also shows a heat exchanger, which the ABI system does not have. 
2	Close the flowcell door, making sure the red tubing is not bent. The yellow and red tubes should exit through the slit in the microbore head adapter. 
3	Route the tubes through a notch in the chassis on the right side of the detector, as shown in the figure. 
4	Close the drawer of the detector, then tighten the knurled screw on the front panel and the knurled nut on the back of the detector.
5	Place the detector on top of the 433A instrument.

To attach the flowcell: *(continued)*

Step	Action
6	Connect one end of the output cable to the REC 10 mV port and the other end to Channel 2 IN, located on the back, right side of the 433A instrument, as shown in the diagram. Maintain the +/- polarity. **IMPORTANT** Use the REC 10 mV port on the right and not the COMP 1 V port on the left, which is used for the Procise sequencer. 

To attach the flowcell: *(continued)*

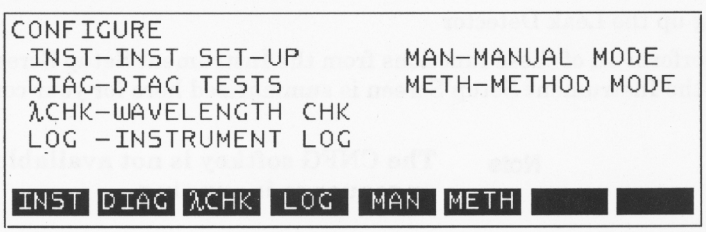
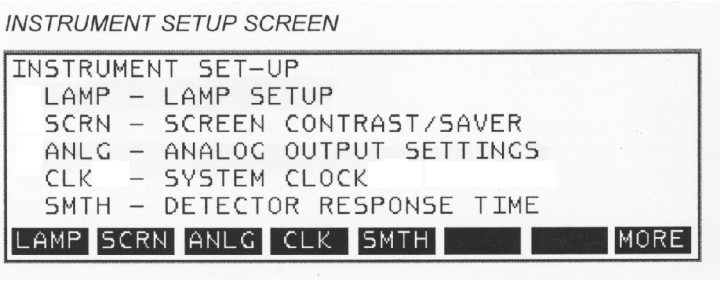
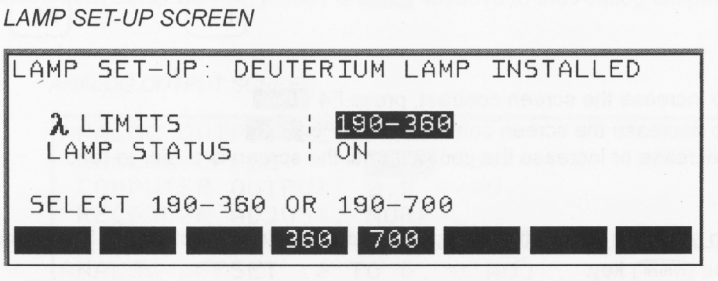
Step	Action
7	Verify that the panel on the right side of the 433A instrument is removed. The auxiliary waste tube to the 11-port (middle) valve block at position 9 (4th port from the top of the middle valve block) is removed and replaced with the end of the red PTFE tube with the valve-port ferrule and bushing. 

Checking for Leaks

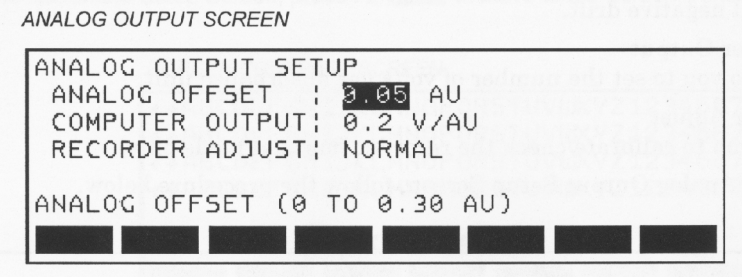
To check for leaks:

1	Open the flowcell compartment.
2	Open the flowcell door.
3	Install a new 450-mL bottle of methanol at position 6.
4	Place the free end of the yellow tube from the flowcell in a beaker.
5	Using manual control, prime the methanol using function 19 for 3 to 4 seconds. Inactivate function 19.
6	Using manual control, open valves 30, 19, 16, and 9 together for 60 seconds. Close the valves when done.
7	Check for leaks around the flowcell (inspect fitting for leaks).
8	If there are no leaks, close the flowcell compartment by following steps 2 to 4 of “Attaching the Flowcell” on page 7-7.

Configuring the Detector

Step	Action
1	Plug in, then power on the detector. In the main screen, press **F7**. The Configure screen opens. *CONFIGURE SCREEN* 
2	Press **F1**. The Instrument Setup screen opens. From this screen you can access the lamp setup and the analog output setting screens. *INSTRUMENT SETUP SCREEN* 
3	Press **F1**. The Lamp Set-Up screen opens. *LAMP SET-UP SCREEN* 
4	Set the wavelength limit to **190** to **700** by pressing the **F5** soft key under the 700 on the screen.
5	To: ♦ Save changes – Press Return to save changes and exit this screen. ♦ Exit without saving – Press Quit to exit the screen. **Note** For changes to take effect, you must press **Return** after editing the setting on any of the screens.

(continued)

Step	Action
6	On the Instrument Setup screen (see step 2), press **F3** to access the Analog Output Screen. On this screen, the analog offset is specified for use with the 433A. The value for the computer output is not important, because the output is taken from the REC output. *ANALOG OUTPUT SCREEN* 
7	Select the Analog Offset field, then enter **0.00**.
8	Press **Enter**. **Note** To adjust the screen contrast, follow the directions on page 6-6 of the *PerkinElmer Series 200 UV/Vis Detector User Manual* (Rev A).

Changing Wavelength, Absorbance and Rise Time Values

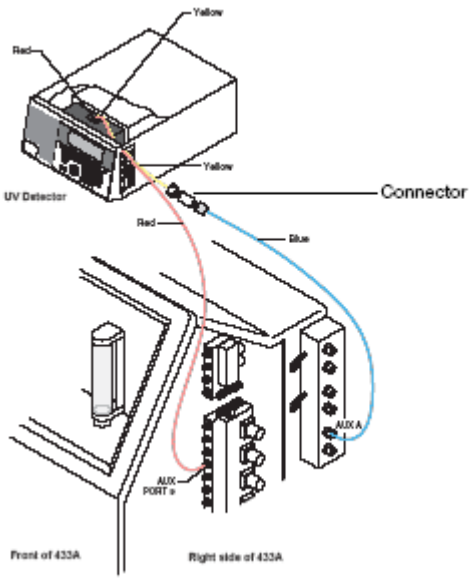
The values for the Wavelength, Range, and RSP (response time in seconds) are changed in the Main set-up screen.

For the 433A instrument:

Wavelength = 301 nm
Range = 0.025 AUF
RSP = 2 seconds

Calibrating the Monitoring System

To calibrate the monitoring system:

Step	Action
1	Send UV flow tests S200 to the 433A instrument.
2	Ensure a beaker is under the yellow tube. Perform module A, which is a 150-sec delivery of NMP through the detector flowcell. Collect the NMP in the beaker.
3	Repeat run module A until there are no bubbles in the lines.
4	Tare the beaker, then run module A again. Collect the NMP in the beaker, then weigh the beaker. **Note** The acceptable range for the NMP flow is 0.75 to 0.85 mL/min, which for a 2.5 min test is 1.94 to 2.19 g NMP. If the NMP flow is too slow, shorten the yellow tube at its end by cutting approximately 2.5 cm (1 in) of yellow tubing at a time. Perform run module A after each cut.
5	When the delivery of NMP for run module A is between 1.94 and 2.19 g, perform a final test by placing the yellow tube into a 10-mL graduated cylinder. Perform module B, which is a 5-min delivery of NMP. The volume of NMP should be 3.75 to 4.25 mL.
6	Attach the threaded connector to the end of the yellow tube, then attach this to the blue PTFE tube (PN 4332158). The other end of the blue tube is attached to the AUX waste port. Refer to the figure below. 

Testing the Monitoring System

Step	Action
1	Unplug the electrical line to the conductivity cell.
2	Remove the cell and the short tube to the in-line filter: connect the “long” tube from the valve block to the in-line filter (PN 603278).

(continued)

Step	Action
3	Perform run Module g (channel 2 re-set) using the Module Test screen on the 433A instrument. Run Module g changes the default monitoring channel from channel 1 (conductivity) to channel 2 (UV). **Note** Whenever a cold reboot occurs, the monitoring channel changes back to channel 1.

Di-Peptide Synthesis

Overview

After the entire startup procedure is complete and all the flowtest specifications are met, synthesis of a standard test peptide is necessary to evaluate instrument performance.

Note The instrument performance functionality will be verified, according to the manufacturer's specifications, by leak tests, chemistry specific flowtests, and the running of a Di-mer (Phe-Gly) on *FastMoc*™ chemistry with Conductivity monitoring or *FastMoc*™ with UV monitoring.

Certain chemicals used in the testing are not supplied by Applied Biosystems but by independent third party vendors therefore results from Conductivity or UV Monitoring may be affected by the quality of the chemicals used in the testing and such effects will be reflected in the background height and number of deprotection peaks obtained for each Amino Acid. The variability in the background height and/or number of Deprotection Peaks is not necessarily an indication of the instrument not performing according to the manufacturer's specifications.

Synthesis to Evaluate Instrument Performance

The results must meet or exceed the minimum installation performance, based on evaluation of the monitoring trace, for the installation to be considered satisfactory. All flow tests previously specified must meet specification prior to performing this synthesis.

As the synthesis begins, check for plumbing leaks, and ensure that the sides of the activator vessel are "sheeting" well when rinsed. (All areas of the vessel should get rinsed by liquid). Watch at least the entire first cycle of the synthesis (first amino acid addition) to observe activation and transfer to the reaction vessel.

Note Because the chemistry kit includes a preloaded resin (Gly), only one coupling cycle is required.

Presynthesis Check

Presynthesis Instrument, Reagent, and Application Check

Before beginning the synthesis of a test peptide, check the equipment, reagents and application.

Step	Action
1	Perform a presynthesis instrument check as follows: a. Check that liquid levels of reagents and solvents are adequate for synthesis. b. Check that the waste container is empty.
2	Obtain: ♦ Necessary amino acid cartridge ♦ A vial of resin ♦ A small-scale (8-mL) reaction vessel

Priming the UV Lines and Zeroing the Detector

Step	Action
1	Run Fxn 101 (MeOH to the AUX) for about 60 sec. Press the **autozero** key on the S200.
2	Using Module Test, run module e (MeOH, NMP to AUX). Watch the 60-sec delivery of MeOH (Fxn 101: User Function B) and the UV absorbance display. When the MeOH has about 5 to 10 sec remaining, press the **autozero** key again on the S200. **Note** Module e may have to be run a few times to be certain that bubbles are cleared from the cell and the zero is stable. **Note** Zeroing the detector with MeOH should give an absorbance reading of 0.05 to 0.10 AU when NMP is running through the flowcell.

Creating the Di-Mer Sequence and Run

Creating the Di-Mer Sequence

Step	Action
1	Select **File > New**. A dialog box opens.
2	Select **Sequence**.
3	Enter the sequence **Phe-Gly**.
4	Save as **Di-mer test**.

Creating a Run for the Synthesis

Step	Action
1	Select **File > New**. A dialog box opens.
2	Select **Run** for File Type. An untitled run file opens on the computer screen.
3	Select **UV *FastMoc*™ 0.1**0 **S200 chemistry**, (previously edited), from the top pop-up window of the run page.
4	From the second pop-up window, create sequence from the Sequence folder.
5	In the Calculations page, click **Amide resin** in the upper-left corner.
6	In the pop-up window, select **Preloaded**, then press the **return** key to save the resin changes in the Calculations window.

Preparing the Resin

Calculating the Amount of Resin Needed for Synthesis

Step	Action
1	Obtain the loading or substitution value in mmol/gram from the resin vial label.
2	Calculate the required weight of resin: a. Enter the substitution value for the resin in the appropriate column. b. Press the **Enter** key to save the change. c. Enter **0.1** in the Resin mmol column. – The calculation sheet automatically calculates the amount of resin you need for synthesis at this 0.1-mmol scale and displays it under “weight g”. – You can also calculate the amount of resin by the formula: 0.1 mmol/substitution value (mmol/g) – Typical substitution values range from 0.6 to 0.9 mmol/gm. For example, a substitution of 0.72 mmol/g would require: 0.1 mmol/ 0.72 mmol/g = 0.139 g or 139 mg of resin
3	**SAVE** the run in the User run folder; it is saved as Run ##.

Sending the Run File to the ABI 433A Synthesizer

Step	Action
1	Select **Synthesizer > Send**. ♦ A chirp sounds when the ABI 433A instrument receives the run file. ♦ A log file is generated automatically when a run file is sent to the instrument.
2	Open the monitor window of the run file to view the deprotection peaks.
3	Save the log file to a log folder: a. Select **File > SAVE AS**. b. Enter a run name or date in the dialog box.
4	Select **File > Auto-SAVE**. The Auto-SAVE feature saves collected data every 5 min. ♦ If power is lost and restored, the Auto-SAVE feature ensures that the last 5 min of collected data before the power outage is available. ♦ The default time is 5 min, but this value can be changed to less than 5 min.

Weighing the Resin

Weigh the required amount of resin into the small-scale reaction vessel (RV).

Step	Action
1	Place a tabbed RV filter (PN 401524) in the opening of the vessel, seating it on the interior ledge of the small scale reaction vessel body: a. Screw the bottom cap over the RV opening with the filter, holding the RV in a vertical position at all times. b. Tighten the cap until the first resistance is felt, wait a few minutes, then resume tightening. **IMPORTANT** DO NOT overtighten the caps to the RV body because the threads will crack.
2	Invert the RV to add the resin: a. Weigh out the required amount of resin into the RV. See “Calculating the Amount of Resin Needed for Synthesis” on page 7-16. b. Install the tabbed filter at the upper ledge of the RV. c. Install the top RV cap assembly. **Note** Each preloaded glycine vial contains enough resin for two syntheses plus some excess.
3	Record the lot number of the preloaded resin, loading, and actual resin weight on the Run Sheet of the Installation Report.

Placing the Reaction Vessel in Its Holder and Installing the Amino Acid

Step	Action
1	Place the assembled RV containing the resin in its holder.
2	Slide the pusher all the way to the left and lock it in place.
3	Remove the foil cap insert to expose the septum on the amino acid cartridge.
4	Record the lot number of the cartridge on the Run Sheet.
5	Place an *empty* cartridge in the guide rail, followed by the Phe amino acid cartridge. **Note** The first amino acid, glycine, is already on the resin.
6	Verify the loading order in the SynthAssist® application by selecting **Cartridges** in the Calculations pop-up window of the Run file.
7	Slowly release the pusher and the cartridge retention bar.

Performing and Evaluating the Run

Starting the Run

Step	Action
1	Return to the Main Menu on the instrument front panel.
2	Select the following in sequence: a. **cycle monitor** b. **Resin Sampling?** – Answer **NO** – This is the default condition and you will normally only need to press **CONTINUE** c. **Hold Times** – Answer **0 (zero)** – This is the default condition and you will normally only need to press **CONTINUE** d. **...print events?** Select **no**. e. **begin**
3	Check for leaks from the RV.
4	View the deprotection peaks to verify that they are of normal height, similar to those in Figure 7-1 on page 7-20.
5	After the run is complete, have the customer save the run and the log file. **Note** The run completes in approximately 1 to 1.5 hours.

Passing Criteria for the Run

Passing criteria for the run are based on the evaluation of the monitoring data. An example is shown in Figure 7-1 on page 7-20. The criteria are based on the number of deprotection peaks observed for each residue of the peptide.

Using Table 7-1, the example in Figure 7-1 meets the passing criteria because the number of deprotection peaks observed are within the range of those expected.

Table 7-1 Expected number of deprotection peaks for each residue for UV installations

Cycle	Residue	Minimum Number of Deprotection Peaks for Each Residue
1	Gly (Preloaded)	2
2	Phe	2

Examples of Deprotection Peak Criteria

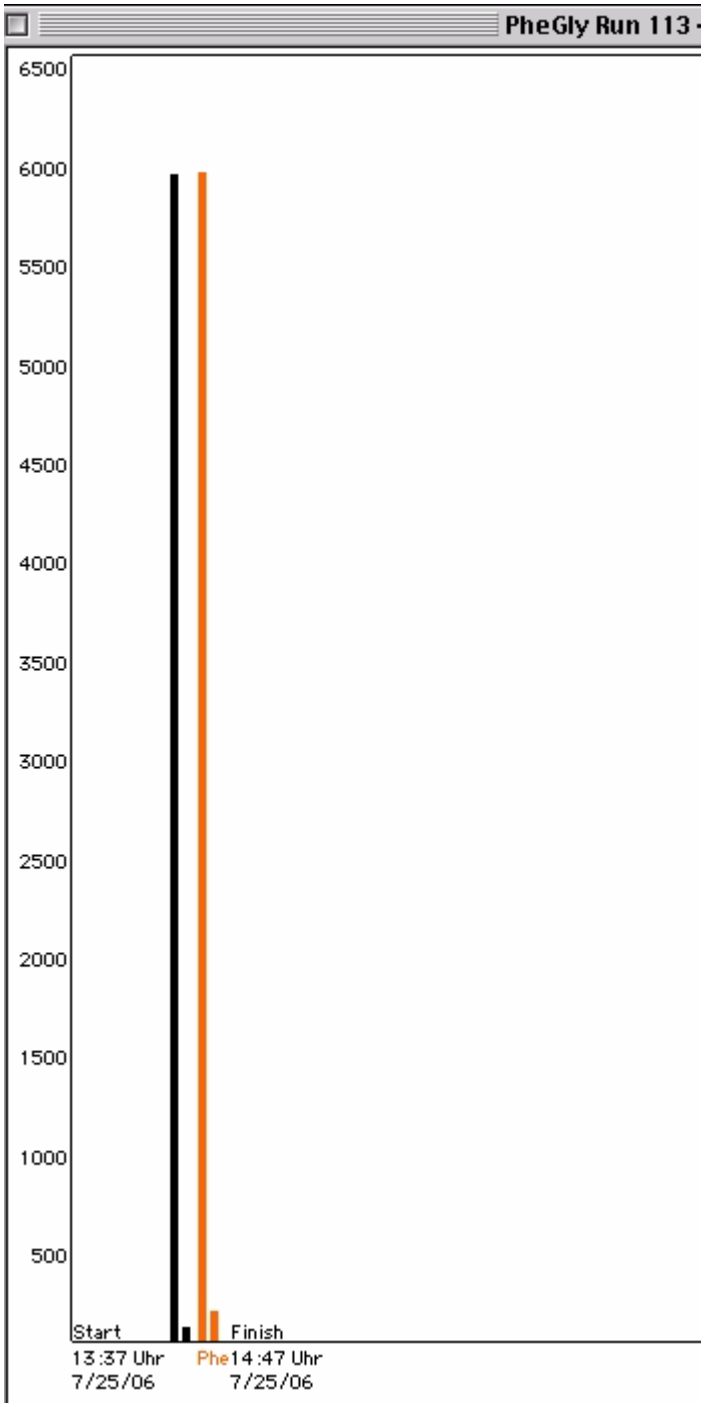
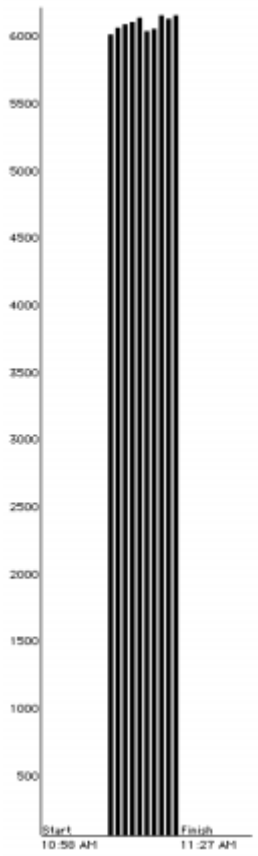
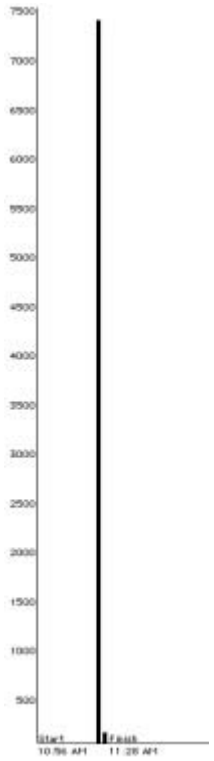


Figure 7-1 Example of deprotection peak criteria for determining a successful run

Performing the Fmoc-Phe Test

Step	Action
1	Open the Fmoc-Phe-OH cartridge provided with the kit. **Note** The cartridge contains approximately 388 mg of Fmoc-Phe-OH.
2	Add approximately 100 mg of Fmoc-Phe-OH to the 40-mL RV.
3	Start a New/Run using the values: ♦ Sequence = **None** ♦ Cycle = **UV Fmoc–Phe Test**
4	Start the synthesis and open the monitoring window. This test checks for timing and consistency of peak heights. Peaks should have similar values but not necessarily have identical heights. The expected monitor display is ten peaks, all approximately the same height, at about 5000 to 6000 units. A typical Fmoc-Phe Test UV Monitor Trace is shown below. 

Performing a Resin Deprotection Test

Step	Action
1	Send the S200 UV *FastMoc*™ 0.10 cycles to the 433A.
2	Start a New/Run: ♦ Sequence = **none** Cycles = **Fmoc–Gly Resin Test**
3	Weigh 0.10 mmol of Fmoc-Gly resin (PN 400847) into the 8-mL RV.
4	Start the synthesis and open the monitoring window. The second deprotection peak for each cycle should be 3.5% or less than the first deprotection peak. A typical resin deprotection monitor trace is shown below. 

Completing the *FastMoc*™ Chemistry Installation

Complete Installation

FastMoc™ chemistry installation is complete if:

- The data values and all flow test results meet or exceed the minimum values required.
- Customer training is complete, that is, the customer has been trained in:
  - Theory
  - Operation
  - Maintenance
- The warranty policy has been explained to the customer.
- All paperwork is complete and the customer has signed the:
  - Service Order
  - Installation Report

Have the customer discard both the finished product from the RV and the used RV filters in accordance with information in the MSDS and waste profile.

Flow Tests

A

Flow Test Descriptions

About Flow Tests

Each flow test consists of a sequence of timed steps, written as a short module in SynthAssist® Software. Explanations and printouts of each flow test are found in this section. Flow tests are used to:

- Adjust the gas regulators
- Flush chemicals through the lines
- Check for proper chemical flows
- Troubleshoot the instrument

There are 25 flow tests, in two SynthAssist® software folders. Flow Tests 1-18 are listed in Table A-1.

Table A-1 Flow Tests 1-18

Flow Test Number	SynthAssist® Software Module	Description
1	a	Bottle 1 to RV
2	b	Bottle 2 to RV
3	c	Barcode reader
4	d	Bottle 4 to RV
5	e	Bottle 5 to RV
6	f	Bottle 6 to RV
7	g	Bottle 7 to waste
8	h	Bottle 8 to waste
9	i	Bottle 9 to RV
10	A	Bottle 10 to RV
11	B	Bottle 10 to cartridge
12	C	Bottle 9 to cartridge
13	D	Bottle 5 to cartridge
14	E	Bottle 10 to cartridge, ACT and RV
15	F	Bottle 9 to top ACT with drain
16	G	Bottle 10 to top of RV
17	H	Measure Bottle 7 to cartridge

Flow Test Number	SynthAssist® Software Module	Description
18	l	Measure Bottle 8 to cartridge

When performing most flow tests, you must place a metering vessel (P/N 400256) in the RV holder. Six flow tests (11, 12, 13, 14, 17 and 18) require an empty, preweighed, septum-sealed cartridge placed in the autosampler.

IMPORTANT To prevent accidental chemical spills, put an empty, septum-sealed cartridge in the guideway and place a pressure block against it before starting flow tests 11, 12, 13, 14, 17 and 18.

Flow Test 19 requires an RV with the resin sample (RS) tube attached to the RS bulkhead and a test tube to collect the RS.

Flow Test 22 requires an 8-mL reaction vessel with filters in place. Flow Test 23 requires a 41 mL reaction vessel with filters in place. Flow Tests 19 to 23 are listed in Table A-2.

Table A-2 Flow Tests 19-23

Flow Test Number	SynthAssist® Software Module	Description
19	a	Resin sample
20	b	Conductivity, Bottles 1, 6, 9, 10
21	c	Chnl 2, 100 mg Fmoc-Phe-resin
22	d	Conductivity baseline, 0.10 mmol
23	e	Conductivity baseline, 0.25 mmol

Table A-3 UV Flow Tests

Flow Test Name	SynthAssist® Software Module	Description
UV Fmoc-Phe Test	e, a, b, d	Fmoc removal, UV signal, solution timing
UV Fmoc Resin Test	e, c, D, H, D, c	Resin sample

Some flow tests should be performed before every synthesis. Table A-3 lists these flow tests, according to the chemistry option you choose for your synthesis, in the order in which they should be performed.

Table A-4 Flow Tests Performed Before Each Synthesis

Chemistry Option	Flow Test
FastMoc™	10, 11, 1, 13, 17
Fmoc/HOBt/DCC	10, 11, 1, 17, 18
Fmoc Loading	4, 18
Boc/HOBt/DCC	10, 11, 2, 1, 17, 18

Use Flow Tests 10 and 11 to adjust the lower regulators. Use Flow Test 2 to adjust the upper regulator. Use Flow Tests 1, 4, 5, 6, and 9 to check the reagent flows. Flow Tests 7 and 8 should be run before a synthesis whenever the instrument has not been used for 1 or more weeks.

Flow Test 1 (Flow Tests 1-18, module a)

Purpose: To check for proper flow of Bottle 1 and to flush the delivery line.

Delivery: 5-sec delivery of the contents of Bottle 1 to the metering vessel in the RV holder.

Expected Results:*FastMoc*™ chemistry or Fmoc/HOBt/DCC: Piperidine, 0.80 to 1.30 mL Boc/HOBt/DCC: DIEA, 1.9 to 2.4 mL

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Note After delivery and measurement, the metering vessel is washed with NMP. It is not washed with DCM since a solution of piperidine and DCM slowly reacts to form crystals of piperidine hydrochloride.

Table A-5 Flow Test 1

Step #	Fx	Fxn Name	Time (sec)
1	13	#10 T VB	1
2	14	#10BVB	1
3	79	PRS#1	15
4	16	#1B YB	3
5	9	GAS TVB	2
6	42	DRAIN RV	5
7	10	GAS B VB	2
8	51	#1 B RV	5
9	40	MIX RV	3
10	1	WAIT	10
11	42	DRAIN RV	10
12	10	GAS B VB	2
13	56	#10 B RV	10
14	40	MIX RV	3
15	42	DRAIN RV	5
16	50	#10 RV-DRN	5
17	42	DRAIN RV	10
18	13	#10 T VB	1
19	14	#10 B VB	1
20	9	GAS T VB	2
21	10	GAS B VB	2
22	98	BEGIN LOOP	2
23	2	VORTEX ON	1
24	56	#10BRV	20
25	40	MIX RV	2
26	3	VORTEX OFF	1

Table A-5 Flow Test 1 *(continued)*

Step #	Fx	Fxn Name	Time (sec)
27	42	DRAIN RV	20
28	99	END LOOP	1
29	13	#10 T VB	1
30	14	#10 B VB	1
31	9	GAS T VB	10
32	10	GAS B VB	10

Flow Test 2 (Flow Tests 1-18, module b)

Note This flow test is not necessary when the ABI 433A instrument is using *FastMoc*™ chemistry or Fmoc/HOBt/DCC chemistry.

Purpose: To set the upper regulator pressure, to check Bottle 2 delivery, and to check for leaks in the TFA seal.

Flow Test 2 only measures TFA once. To readjust the regulator or to repeat a delivery measurement, return to step 1 when the flow test reaches step 29 (wait 60 seconds).

To avoid physical contact with TFA, run the neutralization and washing steps (steps 30 through 68) before removing the metering vessel from the RV holder.

Delivery: 18-sec delivery of the contents of Bottle 2 to the metering vessel in the RV holder.

Expected Results: Boc/HOBt/DCC: TFA, 1.95 to 2.05 mL

FastMoc™ chemistry or Fmoc/HOBt/DCC: no reagent

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

IMPORTANT To prevent over-pressurization of the TFA bottle, the pressure reading on the upper regulator should never exceed 3.5 psi. Typical readings on this regulator, which controls the gas pressure to Bottle 2, is 2.3-3.0 psi.

Table A-6 Flow Test 2

Step#	Fxn #	Fxn Name	Time (sec)
1	73	VENT #2	2
2	75	GAS-VENT #2	2
3	73	VENT #2	2
4	2	VORTEX ON	1
5	55	#9 B RV	5
6	40	MIX RV	2
7	3	VORTEX OFF	1
8	42	DRAIN RV	10
9	76	PRS #2	25
10	71	#2 B VB	2
11	10	GAS B VB	2
12	12	#9 B VB	2
13	10	GAS B VB	3

Table A-6 Flow Test 2 *(continued)*

Step#	Fxn #	Fxn Name	Time (sec)
14	72	#2 B RV	18
15	40	MIX RV	2
16	41	VENT RV	2
17	10	GAS B VB	3
18	73	VENT #2	3
19	74	FLUSH #2	3
20	73	VENT #2	3
21	75	GAS-VENT #2	3
22	73	VENT #2	3
23	74	FLUSH #2	3
24	73	VENT #2	3
25	41	VENT RV	2
26	42	DRAIN RV	5
27	49	#9 RV-DRAIN	10
28	42	DRAIN RV	10
29	1	WAIT	60
30	2	VORTEX ON	1
31	55	#9 B RV	5
32	40	MIX RV	2
33	3	VORTEX OFF	1
34	42	DRAIN RV	8
35	11	#9 T VB	2
36	12	#9 B VB	2
37	9	GAS T VB	2
38	10	GAS B VB	2
39	2	VORTEX ON	1
40	55	#9 B RV	15
41	40	MIX RV	1
42	79	PRS #1	10
43	41	VENT RV	2
44	51	#1 B RV	6
45	55	#9 B RV	10
46	15	#1 T VB	1
47	16	#1 B VB	1
48	11	#9 T VB	2
49	12	#9 B VB	2
50	40	MIX RV	10
51	10	GAS B VB	3
52	3	VORTEX OFF	1
53	42	DRAIN RV	20
54	12	#9 T VB	2

Table A-6 Flow Test 2 *(continued)*

Step#	Fxn #	Fxn Name	Time (sec)
55	12	#9 B VB	2
56	9	GAS T VB	2
57	10	GAS B VB	2
58	2	VORTEX ON	1
59	55	#9 B RV	20
60	40	MIX RV	5
61	9	GAS T VB	2
62	10	GAS B VB	2
63	3	VORTEX OFF	1
64	42	DRAIN RV	20
65	11	#9 T VB	2
66	12	#9 B VB	2
67	9	GAS T VB	10
68	10	GAS B VB	10

Flow Test 3 (Flow Tests 1-18, module c)

Purpose: To check barcode reader accuracy.

Expected Results: A printed list with each amino acid cartridge properly identified.

Requirements: A series of cartridges with legible, unmarred barcode labels and a printer.

Step	Action
1	Load the amino acid cartridges.
2	Transfer Flow Tests 1-18 from SynthAssist® and run module c.

Table A-7 Flow test 3

Step#	Fxn #	Fxn Name	Time (sec)
1	98	BEGIN LOOP	99
2	1	WAIT	1
3	4	PRINT CART	10
4	7	EJECT CART	10
5	8	ADVAN CART	10
6	99	END LOOP	1

Flow Test 4 (Flow Tests 1-18, module d)

Purpose: To check for proper flow of Bottle 4 and to flush the delivery line.

Delivery: 5-sec delivery of the contents of Bottle 4 to the metering vessel in the RV holder.

Expected Results: *FastMoc*™ chemistry or Fmoc/HOBt/DCC: DMAP, 2.0 to 2.30 mL Boc/HOBt/DCC:Ac_2O, 1.70 to 2.30 mL

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Table A-8 Flow Test 4

Step #	Fxn#	Fxn Name	Time (sec)
1	9	GAS T VB	2
2	42	DRAIN RV	3
3	10	GAS B VB	2
4	77	PRS #4	15
5	52	#4 B RV	5
6	40	MIX RV	2
7	1	WAIT	10
8	42	DRAIN RV	7
9	10	GAS B VB	5
10	49	#9 RV-DRN	5
11	42	DRAIN RV	10
12	11	#9 T VB	1
13	12	#9 B VB	1
14	9	GAS T VB	2
15	10	GAS B VB	2
16	2	VORTEX ON	1
17	55	#9 B RV	15
18	40	MIX RV	3
19	3	VORTEX OFF	1
20	42	DRAIN RV	20
21	11	#9 T VB	1
22	12	#9 B VB	1
23	9	GAS T VB	10
24	10	GAS B VB	10

Flow Test 5 (Flow Tests 1-18, module e)

Purpose: To check for proper flow of Bottle 5 with Boc chemistry and to flush the delivery line.

Delivery: 5-sec delivery of contents of Bottle 5 to the metering vessel in the RV holder.

Expected Results: Boc/HOBt/DCC: DMSO, 0.8 to 1.3 mL

FastMoc™ chemistry or Fmoc/HOBt/DCC: Use Flow Test 13.

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Table A-9 Flow Test 5

Step#	Fxn#	Fxn Name	Time (sec)
1	42	DRAIN RV	3
2	10	GAS B VB	2
3	78	PRS #M	15
4	53	#5 B RV	5
5	40	MIX RV	2
6	1	WAIT	10
7	42	DRAIN RV	10
8	50	#10 RV-DRN	15
9	42	DRAIN RV	10
10	49	#9 RV-DRN	20
11	42	DRAIN RV	12
12	10	GAS B VB	3

Flow Test 6 (Flow Tests 1-18, module f)

Purpose: To check for proper flow of Bottle 6 and to flush lines.

Delivery: 5-sec delivery of contents of Bottle 6 to the metering vessel in the RV holder.

Expected Results: *FastMoc*™ chemistry, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: Methanol, 1.95 to 2.55 mL

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Table A-10 Flow Test 6

Step#	Fxn#	Fxn Name	Time (sec)
1	9	GAS T VB	2
2	42	DRAIN RV	3
3	10	GAS B VB	2
4	78	PRS #M	15
5	54	#6 B RV	5
6	40	MIX RV	2
7	9	GAS T VB	10
8	42	DRAIN RV	15
9	10	GAS B VB	10

Flow Test 7 (Flow Tests 1-18, module g)

Purpose: To flush the delivery line of Bottle 7.

Delivery: 4-sec delivery of contents of Bottle 7 to waste.

Expected Results: Boc/HOBt/DCC and Fmoc/HOBt/DCC: 1M HOBt, no measurements are taken. *FastMoc*™ chemistry: 2 M DIEA, no measurements are taken.

Procedure:

Note This flow test should be run before a synthesis if the instrument has not been used for several days to check the line for blockages to Bottle 7.

To check line to Bottle 7 for blockages:

Step	Action
1	Place a metering vessel in the RV holder. **Note** No reagent is delivered to the metering vessel during Flow Test 7.
2	Remove the right side panel.
3	Run Flow Test 7. Watch the 0.5 mL loop fill up during the flow test.
4	**If the 0.5 mL loop...** / **Then...** fills up during the first 3 sec of step 2 / ♦ A blockage does not exist in the line to Bottle 7. ♦ Proceed to perform Flow Test 7. does not fill in 3 sec / ♦ A crystalline precipitate may be blocking the line to Bottle 7. ♦ Proceed to dissolve the crystals.

To dissolve the crystals:

Step	Action
1	Replace Bottle 7 with a bottle of DCM/MeOH (60/40).
2	Run Flow Test 7 again.
3	At step 2, press **hold** until NMP flows easily through the loop.
4	Remove the NMP bottle and replace it with an empty bottle.
5	Repeat Flow Test 7 once more to let nitrogen flow through the loop.
6	Clean the outside of the tubing with a lint-free tissue and replace Bottle 7.
7	Repeat Flow Test 7.
8	**If the 0.5 mL loop...** / **Then...** fills up during the first 3 seconds of step 2 / ♦ The line to Bottle 7 is clear. ♦ Proceed to perform Flow Test 7. does not fill in 3 seconds / ♦ Check the metering loop and the attached tubing for crimps or leaks. ♦ Check for leaks in the tubing associated with Bottles 5 through 8. ♦ See the User Guide for a more detailed washing procedure.

Table A-11 Flow Test 7

Step #	Fxn #	Fxn Name	Time (sec)
1	78	PRS #M	15
2	68	MEAS #7	4
3	70	PURGE ML	5
4	10	GAS B VB	2
5	14	#10 B VB	2
6	10	GAS B VB	10

Flow Test 8 (Flow Tests 1-18, module h)

Purpose: To flush the delivery line for Bottle 8.

Delivery: 4-sec delivery of contents of Bottle 8 to waste.

Expected Results: *FastMoc*™ chemistry, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: 1M DCC, no measurements are taken.

Procedure: Similar to Flow Test 7.

Note This flow test should be run before a synthesis if the instrument has not been used for several days to check the line for blockages to Bottle 8.

To check line to Bottle 8 for blockages:

Step	Action
1	Place a metering vessel in the RV holder. **Note** No reagent is delivered to the metering vessel during Flow Test 8.
2	Remove the right side panel.
3	Run Flow Test 8. Watch the 0.5 mL loop fill up during the flow test.
4	**If the 0.5 mL loop...** / **Then...** fills up during the first 3 sec of step 2 → ♦ A blockage does not exist in the line to Bottle 8. ♦ Proceed to perform Flow Test 8. does not fill in 3 sec → ♦ A crystalline precipitate may be blocking the line to Bottle 8. ♦ Proceed to dissolve the crystals.

To dissolve the crystals:

Step	Action
1	Replace Bottle 8 with a bottle of DCM/MeOH (60/40).
2	Run Flow Test 8 again.
3	At step 2, press **hold** until NMP flows easily through the loop.
4	Remove the NMP bottle and replace it with an empty bottle.
5	Repeat Flow Test 8 once more to let nitrogen flow through the loop.
6	Clean the outside of the tubing with a lint-free tissue and replace Bottle 8.
7	Repeat Flow Test 8.
8	**If the 0.5 mL loop...** / **Then...** fills up during the first 3 sec of step 2 → ♦ The line to Bottle 8 is clear. ♦ Proceed to perform Flow Test 8. does not fill in 3 sec → ♦ Check the metering loop and the attached tubing for crimps or leaks. ♦ Check for leaks in the tubing associated with Bottles 5 through 8. ♦ See the User Guide for a more detailed washing procedure.

Table A-12 Flow Test 8

Step #	Fxn #	Fxn Name	Time (sec)
1	78	PRS #M	15
2	69	MEAS #8	4
3	70	PURGE ML	5
4	10	GAS B VB	2
5	14	#10 B VB	2
6	10	GAS B VB	10

Flow Test 9 (Flow Tests 1-18, module i)

Purpose: To check for proper flow of Bottle 9 and to flush the delivery line.

Delivery: 5-sec delivery of contents of Bottle 9 to the metering vessel in the RV holder.

Expected Results: *FastMoc*™ chemistry, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: DCM, 2.8 to 3.5 mL

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Table A-13 Flow Test 9

Step#	Fxn#	Fxn Name	Time (sec)
1	9	GAS T VB	2
2	42	DRAIN RV	3
3	10	GAS B VB	2
4	55	#9 B RV	5
5	40	MIX RV	3
6	9	GAS T VB	10
7	42	DRAIN RV	10
8	10	GAS B VB	10

Flow Test 10 (Flow Tests 1-18, module A)

Purpose: To set lower regulator pressure and to flush line.

Delivery: 5-sec delivery of contents of Bottle 10 to the metering vessel in the RV holder.

Expected Results: *FastMoc*™ chemistry, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: NMP, 2.4 to 2.6 mL* or 2.5mL ± 0.10mL.

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Note The lower regulator monitors gas to the valve blocks and pressure to all the chemical bottles, except Bottle 2. The pressure on the lower regulator should never be permitted to go above 11 psi.

Table A-14 Flow Test 10

Step#	Fxn#	Fxn Name	Time (sec)
1	9	GAS T VB	2
2	42	DRAIN RV	3
3	10	GAS B VB	2
4	56	#10 B RV	5
5	40	MIX RV	3
6	9	GAS T VB	10
7	42	DRAIN RV	10
8	10	GAS B VB	10

* If the pressure on the lower regulator has been increased to assure a 2.0 g delivery of Bottle 10 in Flow Test 11, the delivery in Flow Test 10 may be as much as 2.75 mL. However, before increasing the pressure on the lower regulator, first change the cartridge inline filter and check the needle for blockage.

Flow Test 11 (Flow Tests 1-18, module 1B)

Purpose: To check for proper flow from Bottle 10 and from the inline filter to cartridge.

Delivery: 5-sec delivery of contents of Bottle 10 to cartridge.

Expected Results:*FastMoc*™ chemistry, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: NMP, 1.95 to 2.35 grams

Requirements: Preweighed cartridge

To check flow from Bottle 10 and from the inline filter to the cartridge:

Step	Action
1	Place preweighed cartridge in autosampler.
2	Run Flow Test 11.
3	Reweigh ejected cartridge to verify expected weight of reagent delivered to cartridge.

Note If the flow to the cartridge is low and Flow Test 10 is correct, replace the inline filter to the cartridge and check the needle for blockage. If the delivery is still inadequate, you may increase the pressure on the lower regulator. However, the pressure on the lower regulator should not exceed 11 psi.

Table A-15 Flow Test 11

Step#	Fxn#	Fxn Name	Time (sec)
1	8	ADVAN CART	10
2	5	NEEDLE DWN	10
3	10	GAS B VB	2
4	65	#10 CART	5
5	60	MIX CART	5
6	6	NEEDLE UP	10
7	7	EJECT CART	10
8	8	ADVAN CART	10
9	10	GAS B VB	10

Flow Test 12 (Flow Tests 1-18, module C)

Purpose: To check for proper flow from Bottle 9 and from the inline filter to cartridge.

Delivery: 5-sec delivery of contents of Bottle 9 to cartridge.

Expected Results: *FastMoc*™ chemistry, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: DCM, 3.40 to 4.10 grams

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Note If the flow to the cartridge is low, and Flow Test 9 is correct, the inline filter to the needle may need replacing.

Table A-16 Flow Test 12

Step#	Fxn#	Fxn Name	Time (sec)
1	8	ADVAN CART	10
2	5	NEEDLE DWN	10
3	10	GAS B VB	2
4	64	#9 CART	5
5	60	MIX CART	5
6	6	NEEDLE UP	10
7	7	EJECT CART	10
8	8	ADVAN CART	10
9	10	GAS B VB	10

Flow Test 13 (Flow Tests 1-18, module D)

Purpose: To determine correct delivery time for HBTU solution to the cartridge. Perform this flow test each time you change either the inline filter to the amino acid cartridge or the filter on the end of the Bottle 5 delivery line. After performing Flow Test 13, perform Flow Test 11 to rinse HBTU out of the inline filter.

Delivery: Nominal 8-sec delivery of contents of Bottle 5 to the cartridge. Actual delivery time will be determined by this test and used in setting times in Module A of *FastMoc*™ Cycles.

Expected Results: *FastMoc*™ chemistry: 1.9 to 2.1 g of 0.45 M HBTU solution.

Requirement: Preweighed cartridge.

To determine the delivery time for HBTU solution to get to the cartridge:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Place the preweighed cartridge in the autosampler.</td></tr>
<tr><td>2</td><td>Run Flow Test 13</td></tr>
<tr><td>3</td><td>Reweigh ejected cartridge to verify expected weight of reagent delivered to cartridge

<table><tr><th>If...</th><th>Then...</th></tr><tr><td>the weight is not 1.9 to 2.1 g</td><td>use Module Editor to adjust the time in step 4 Fxn 94 (#5 TO CART) so that the delivery weight falls within the range.

Note Fxn 94 occurs in module A in each of the FastMoc™ chemistry cycles. Change the time for Fxn 94 in any FastMoc™ chemistry cycles you use until this flowtest is performed again.</td></tr><tr><td>the time exceeds 13 sec</td><td>it is recommended you replace the HBTU filter and repeat this test.</td></tr></table></td></tr>
<tr><td>4</td><td>IMPORTANT After performing each Flow Test 13, Flow Test 11 must then be performed to rinse HBTU out of the inline filter.

Perform Flow Test 11.</td></tr>
</table>

Table A-17 Flow Test 13

Step#	Fxn#	Fxn Name	Time
1	5	NEEDLE DWN	10
2	10	GAS B VB	2
3	78	PRS #M	15
4	94	#5 TO CART	8
5	60	MIX CART	5
6	61	VENT CART	3
7	6	NEEDLE UP	10
8	7	EJECT CART	10

Table A-17 Flow Test 13 *(continued)*

Step#	Fxn#	Fxn Name	Time
9	8	ADVAN CART	10
10	10	GAS B VB	10

Flow Test 14 (Flow Tests 1-18, module E)

Purpose: Use for instrument test and troubleshooting.

Delivery:5-sec delivery of contents of Bottle 10 to the Activator (ACT), then to the metering vessel. This is followed by a 5-second delivery of the contents of Bottle 10 to the cartridge, which is transferred to the ACT, and finally to the metering vessel.

Expected Results: At step # 10, *FastMoc*™ chemistry, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: NMP, 2.00 to 2.80 mL

At step 28, *FastMoc*™ chemistry, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: NMP, minimum 1.00 mL

Requirements: Place a metering vessel in the RV holder. Place a cartridge in the autosampler. Measure the volume of reagents delivered to the metering vessel.

Note This test is not performed routinely before a synthesis.

Table A-18 Flow Test 14

Step#	Fxn#	Fxn Name	Time (sec)
1	9	GAS T VB	2
2	22	DRAIN ACT	3
3	42	DRAIN RV	3
4	10	GAS B VB	2
5	36	#10 B ACT	5
6	20	MIX ACT	2
7	28	GAS T ACT	3
8	38	ACT TO RVo	5
9	40	MIX RV	2
10	1	WAIT	10
11	22	DRAIN ACT	15
12	42	DRAIN RV	15
13	5	NEEDLE DWN	10
14	10	GAS B VB	2
15	65	#10 CART	5
16	60	MIX CART	3
17	24	CART TO AC	10
18	61	VENT CART	2
19	6	NEEDLE UP	10
20	7	EJECT CART	10
21	8	ADVAN CART	10
22	9	GAS T VB	2
23	42	DRAIN RV	3

Table A-18 Flow Test 14 *(continued)*

Step#	Fxn#	Fxn Name	Time (sec)
24	10	GAS B VB	2
25	28	GAS T ACT	3
26	38	ACT TO RVo	4
27	40	MIX RV	2
28	1	WAIT	10
29	22	DRAIN ACT	15
30	42	DRAIN RV	15
31	9	GAS T VB	10
32	10	GAS B VB	10

Flow Test 15 (Flow Tests 1-18, module F)

Purpose: Use for instrument quality control, for troubleshooting and for testing the sheeting action of the activator.

Delivery: Contents of Bottle 9 to top of activator with drain.

Expected Results: *FastMoc*™ chemistry, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: DCM, no measurements are taken. Check for proper sheeting action.

Requirements: None

Procedure: Place a metering vessel in the RV holder. No reagent is delivered to the metering vessel during Flow Test 15.

Note This test is not performed routinely before a synthesis.

Table A-19 Flow Test 15

Step#	Fxn#	Fxn Name	Time (sec)
1	9	GAS T VB	2
2	29	#9 ACT-DRN	10
3	22	DRAIN ACT	25
4	9	GAS T VB	10
5	10	GAS B VB	10

Flow Test 16 (Flow Tests 1-18, module G)

Purpose: Use for instrument quality control, and for troubleshooting.

Delivery: Contents of Bottle 10 to top of metering vessel.

Expected Results: Boc/HOBt/DCC, Fmoc/HOBt/DCC, and *FastMoc*™ chemistry: NMP, 0.90 to 1.30 mL.

Requirements: Place a metering vessel in the RV holder. Measure the volume of reagents delivered to the metering vessel.

Note This test is not performed routinely before a synthesis.

Table A-20 Flow Test 16

Step#	Fxn#	Fxn Name	Time (sec)
1	9	GAS T VB	2
2	42	DRAIN RV	3
3	41	VENT RV	5
4	46	#10 T RV	5
5	48	GAS T RV	3
6	41	VENT RV	2
7	1	WAIT	10
8	42	DRAIN RV	15
9	9	GAS T VB	10
10	10	GAS B VB	10

Flow Test 17 (Flow Tests 1-18, module H)

Purpose: Use to calibrate delivery of Bottle 7 through 0.5 mL loop.

Delivery: 0.5 mL metering loop is filled from Bottle 7 and delivered to cartridge.

Expected Results: Instrument calibration: NMP, 0.493 to 0.554 g

FastMoc™ chemistry: 2M DIEA, 0.42 to 0.50 g

Fmoc/HOBt/DCC: 1M HOBt/NMP, 0.52 to 0.55 g

Boc/HOBt/DCC: 1M HOBt/NMP, 0.52 to 0.55 g

Requirements: Preweighed cartridge.

Procedure: See Flow Test 11.

Note This test is not performed routinely before a synthesis. However, if the instrument has not been used for several days, this flow test or Flow Test 7 should be performed to ensure proper delivery of reagents.

Table A-21 Flow Test 17

Step#	Fxn#	Fxn Name	Time (sec)
1	8	ADVAN CART	10
2	5	NEEDLE DWN	10
3	78	PRS #M	15
4	68	MEAS #7	3
5	10	GAS B VB	2
6	63	ML TO CART	5
7	61	VENT CART	2
8	6	NEEDLE UP	10
9	7	EJECT CART	10
10	8	ADVAN CART	10

Flow Test 18 (Flow Tests 1-18, module I)

⚠ WARNING CHEMICAL HAZARD. Dicyclohexylcarbodiimide (DCC) can cause severe allergic reactions in sensitive people. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

IMPORTANT When running this flow test with 1M DCC in NMP, use appropriate safety precautions. Wear gloves and clean the used cartridge in a well-ventilated hood.

Purpose: Use to calibrate delivery of Bottle 8 through 0.5 mL loop.

Delivery: 0.5 mL metering loop is filled from Bottle 8 and delivered to cartridge.

Expected Results: *FastMoc*™ chemistry, Fmoc/HOBt/DCC, and Boc/HOBt/DCC: 1 M DCC/NMP, 0.515 to 0.554 g; NMP: 0.52 to 0.54 g

Procedure: See Flow Test 11.

Note This test is not performed routinely before a synthesis. However, if the instrument has not been used for several days, this flow test or Flow Test 8 should be performed to insure proper delivery of DCC.

Table A-22 Flow Test 18

Step#	Fxn#	Fxn Name	Time (sec)
1	8	ADVAN CART	10
2	5	NEEDLE DWN	10
3	78	PRS #M	15
4	69	MEAS #8	3
5	10	GAS B VB	2
6	63	ML TO CART	5
7	61	VENT CART	2
8	6	NEEDLE UP	10
9	7	EJECT CART	10
10	8	ADVAN CART	10

Flow Test 19 (Flow Tests 19-23, module a)

Purpose: Use to troubleshoot resin-sampler valves, switches, and the resin-sampler line with Bottle 10.

Delivery: A solution for resin sampling is delivered to a test tube.

Requirements: Resin sample test tube and reaction vessel with resin-sampling line connected to a bulkhead. If you want to check resin sample delivery, fill the RV with approximately 100 mg of resin. Be sure that filters are in place.

Procedure: See Flow Test 1.

IMPORTANT Do not use the metering vessel with this flow test. Use a reaction vessel with the resin-sampling line connected to a bulkhead.

Table A-23 Flow Test 19

Step#	Fxn#	Fxn Name	Time (sec)
1	56	#10 B RV	14
2	41	VENT RV	1
3	88	RS TO RV	1
4	91	#10 TO RS	4
5	89	RS TO FC	1
6	2	VORTEX ON	1
7	40	MIX RV	2
8	91	#10 TO RS	4
9	88	RS TO RV	1
10	93	GAS TO RS	2
11	91	#10 TO RS	4
12	41	VENT RV	2
13	91	#10 TO RS	1
14	48	GAS T RV	2
15	87	TAKE SAMPL	2
16	89	RS TO FC	1
17	90	#9 TO RS	1
18	93	GAS TO RS	2
19	90	#9 TO RS	1
20	93	GAS TO RS	5
21	41	VENT RV	2
22	88	RS TO RV	1
23	90	#9 TO RS	4
24	89	RS TO FC	1
25	39	RELAY 0	1
26	3	VORTEX OFF	1
27	42	DRAIN RV	10
28	41	VENT RV	2
29	88	RS TO RV	1
30	93	GAS TO RS	3

Table A-23 Flow Test 19 *(continued)*

Step#	Fxn#	Fxn Name	Time (sec)
31	41	VENT RV	2
32	89	RS TO FC	1
33	42	DRAIN RV	15
34	9	GAS T VB	10
35	10	GAS B VB	10

Flow Test 20 (Flow Tests 19-23, module b)

Purpose: Use to test the conductivity cell.

Delivery: Four deliveries to conductivity cell, from Bottle 6 (MeOH), Bottle 1 (Piperidine), Bottle 10 (NMP) and Bottle 9 (DCM).

Requirements: Conductivity cell and metering vessel, reagents for *FastMoc*™ chemistry or Fmoc/HOBt/DCC chemistry.

Procedure: (Refer to the *SynthAssist® Software Version 3.1 User Guide* (PN 4338849, Rev. B) for procedures related to SynthAssist® Software).

IMPORTANT Press the RETURN key after each change to prevent settings returning to default settings.

To download Flow Tests 19-23, module b from SynthAssist® software to the ABI 433A instrument:

Step	Action
1	Download Flow Test 19-23 Chemistry file from SynthAssist® software to the ABI 433A instrument.
2	Open a new Run file in SynthAssist® software. a. Go to the **File** pull-down menu. b. Select **New**, then select **Run**.
3	Choose **Flow Test 19-23** from the top pop-up screen. Press the RETURN key to save the changes.
4	Choose **None** for sequence.
5	Type **"b"** under Modules on the Cycles Page. Press the **RETURN** key to save the changes.
6	Select **Save As**. a. Save the run as "Flow Test b" and add the date. b. Save the run in the Test Folder.

To send the run to the synthesizer:

Step	Action
1	**Send** the run to the ABI 433A Peptide Synthesizer. The SynthAssist® software Log window automatically appears.
2	Place a metering vessel in the reaction vessel position on the ABI 433A instrument.
3	Go to "Cycle Monitor" on the ABI 433A instrument.
4	Select **No** to "Resin sampling?".
5	Select **Yes** to "Print run events?".

To send the run to the synthesizer:

Step	Action
6	Select **Begin**.
7	In the SynthAssist® software, open the Monitor window from the Flow Test “b” run file. The monitoring window should display four peaks for each solvent delivery, as shown in Figure A-1.

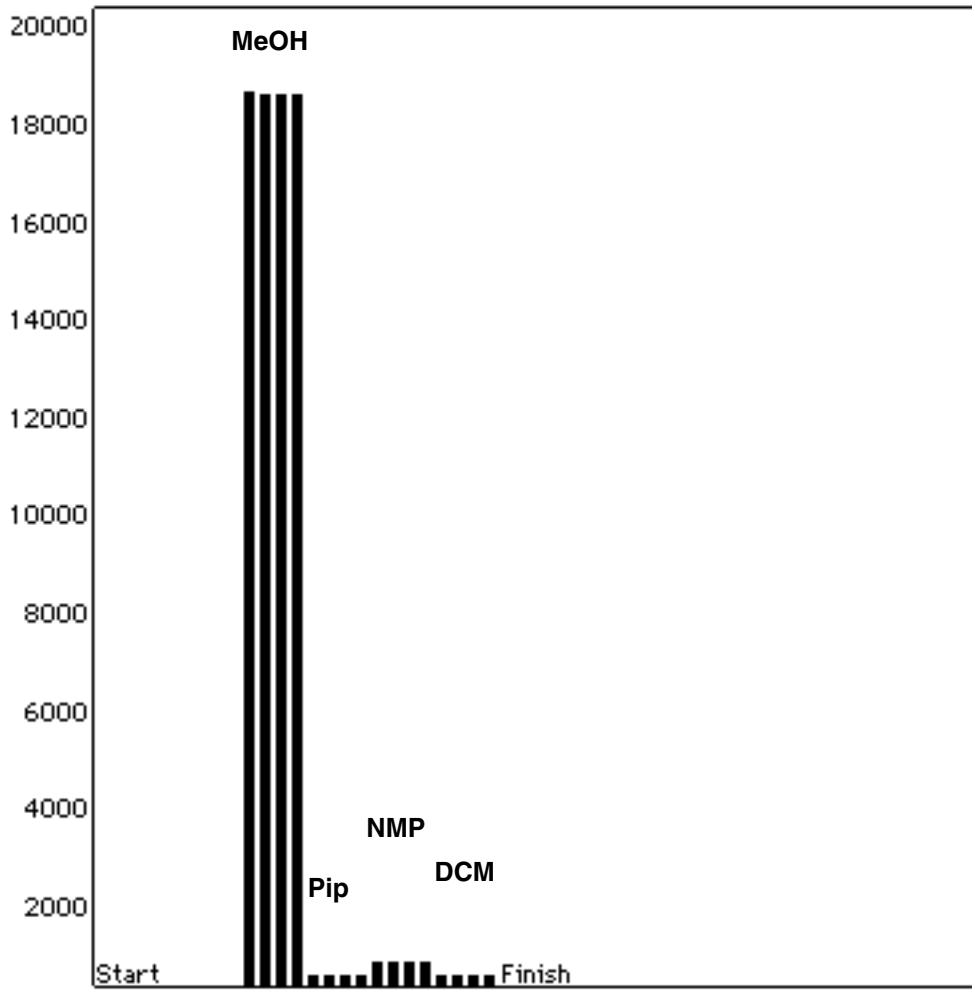


Figure A-1 Monitoring window display of Flow Test 20

Table A-24 Flow Test 20

Step#	Fxn#	Fxn Name	Time
1	1	WAIT	2
2	78	PRS #M	10
3	19	#6 B VB	5
4	98	BEGIN LOOP	4
5	135	Mon Reset	1

Table A-24 Flow Test 20 *(continued)*

Step#	Fxn#	Fxn Name	Time
6	54	#6 B RV	5
7	130	Mon PrevPk	5
8	131	Mon Stop	1
9	132	Save MonPk	1
10	42	DRAIN RV	15
11	99	END LOOP	1
12	79	PRS #1	10
13	16	#1 B VB	5
14	98	BEGIN LOOP	4
15	135	Mon Reset	1
16	51	#1 B RV	5
17	130	Mon PrevPk	5
18	131	Mon Stop	1
19	132	Save MonPk	1
20	42	DRAIN RV	10
21	99	END LOOP	1
22	14	#10 B VB	5
23	98	BEGIN LOOP	4
24	135	Mon Reset	1
25	56	#10 B RV	5
26	130	Mon PrevPk	5
27	131	Mon Stop	1
28	132	Save MonPk	1
29	42	DRAIN RV	10
30	99	END LOOP	1
31	12	#9 B VB	5
32	98	BEGIN LOOP	4
33	135	Mon Reset	1
34	55	#9 B VB	5
35	130	Mon PrevPk	5
36	131	Mon Stop	1
37	132	Save MonPk	1
38	42	DRAIN RV	10
39	99	END LOOP	1
40	9	GAS T VB	5
41	10	GAS B VB	5
42	41	VENT RV	10

Flow Test 21 (Flow Tests 19-21, module c)

Purpose: Use to test Channel 2.

Delivery: Five samples are taken from RV and sent to spectrophotometric source.

Requirements: Standard scale reaction vessel (0.25 mmol), reagents for *FastMoc*™ chemistry or Fmoc/HOBt/DCC chemistry, and 100 mg Fmoc-Phe (pre-loaded resin).

To prepare to perform Flow Test 21:

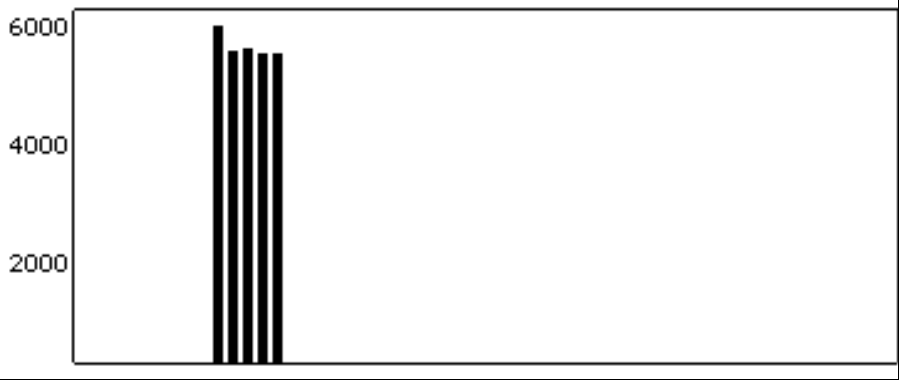
Step	Action
1	Transfer Flow Tests 19-21 from SynthAssist® software to the ABI 433A instrument.
2	On SynthAssist® software, open a new Run.
3	Choose **none** for sequence and save the Run.
4	**Send** the run to the synthesizer.
5	Open the monitor window
6	Place approximately 100 mg Fmoc-Phe resin in the reaction vessel.
7	Place the reaction vessel on the ABI 433A instrument.
8	In the **Module Test** menu, run module c. The monitor window for Flow Test 21 should display 5 peaks, as shown below. 

Table A-25 Flow Test 21

Step#	Fxn#	Fxn Name	Time
1	56	#10 B RV	14
2	79	PRS #1	10
3	51	#1 B RV	8
4	56	#10 B RV	4
5	40	MIX RV	3
6	98	BEGIN LOOP	2
7	2	VORTEX ON	15
8	3	VORTEX OFF	15
9	99	END LOOP	1
10	135	Mon Reset	2
11	98	BEGIN LOOP	5
12	2	VORTEX ON	1
13	14	#10 B VB	2
14	118	#10 TO AUX	5
15	3	VORTEX OFF	1
16	118	#10 TO AUX	15

Table A-25 Flow Test 21 *(continued)*

Step#	Fxn#	Fxn Name	Time
17	42	DRAIN RV	2
18	13	#10T VB	2
19	116	RELAY 1 ON	1
20	129	Mon 1stPk	1
21	118	#10 TO AUX	45
22	131	Mon Stop	1
23	132	Save MonPk	2
24	117	RLY 1 OFF	1
25	10	GAS B VB	4
26	40	MIX RV	2
27	99	END LOOP	1
28	42	DRAIN RV	15
29	98	BEGIN LOOP	2
30	56	#10 B RV	10
31	40	MIX RV	2
32	2	VORTEX ON	5
33	3	VORTEX OFF	1
34	42	DRAIN RV	10
35	50	#10 RV-DRN	5
36	42	DRAIN RV	10
37	99	END LOOP	1
38	49	#9 RV-DRN	10
39	42	DRAIN RV	15
40	9	GAS T VB	3
41	10	GAS B VB	3

Flow Test 22 (Flow Tests 19-23, module d)

Purpose: Use to determine the conductivity baseline of a solution of NMP and piperidine for *FastMoc*™ chemistry 0.10 mmol modules.

Delivery: Five samples are taken from RV and sent to conductivity flow cell.

Requirements: Small scale (8 mL) reaction vessel (0.10 mmol), and reagents for *FastMoc*™ chemistry.

Procedure:

IMPORTANT Press the RETURN key after each change to prevent settings returning to default settings.

To download Flow Tests19-23 from SynthAssist® software to the ABI 433A instrument:

Step	Action
1	Download Flow Tests 19-23 Chemistry file from SynthAssist® software to the ABI 433A instrument.
2	Open a new Run file in SynthAssist® software: a. Go to the **File** pull-down menu. b. Select **New**, then select **Run**.
3	Choose **Flow Test 19-23** from the top pop-up screen. Press the **RETURN** key to save the changes.
4	Choose **None** for sequence.
5	Type "**dd**" under Modules on the Cycles Page. Press the RETURN key to save these changes. **Note** Typing "dd" programs the instrument to perform the run twice.
6	Select **Save As**. a. Save the run as "Flow Test d" and add the date. b. Save the run in the Test Folder.

To send the run to the synthesizer:

Step	Action
1	**Send** the run to the ABI 433A Peptide Synthesizer. The SynthAssist® software Log window automatically appears.
2	Open the **Monitor** window. The conductivity peaks appear in the window once the run begins.
3	Place the 0.10 mmol reaction vessel, with filters, on the ABI 433A instrument.
4	Go to "Cycle Monitor" on the ABI 433A instrument.
5	Select **No** to "Resin sampling?".
6	Select **Yes** to "Print run events?".
7	Select **Begin**.
8	The Log window displays the conductivity of each of five samples of the NMP/piperidine solution. Use the value of the last peak for the conductivity baseline. a. Divide this value by ten. b. Enter this value for "T" in Function 128 when it appears in the *FastMoc*™ 0.10 mmol module B-"Deprotection/Ω Mon 1st Peak-X."
9	Verify similar peak height values and record the value from the second run.

Table A-26 Flow Test 22

Step#	Fxn#	Fxn Name	Time (sec)
1	1	WAIT	1
2	58	INTERRUPT	
3	135	Mon Reset	1
4	133	MonBegLoop	5
5	42	DRAIN RV	10
6	98	BEGIN LOOP	1
7	56	#10 TO RV	5
8	40	MIX RV	2
9	2	VORTEX ON	5
10	3	VORTEX OFF	1
11	42	DRAIN RV	8
12	99	END LOOP	1
13	56	#10 TO RV	3
14	79	PRS #1	10
15	51	#1 TO RV	5
16	56	#10 TO RV	4
17	40	MIX RV	2
18	2	VORTEX ON	20
19	3	VORTEX OFF	1
20	40	MIX RV	1
21	130	MonPrevPk	1
22	42	DRAIN RV	3
23	131	MonStop	1
24	132	Save Mon	1
25	134	MonEndLoop	1
26	42	DRAIN RV	10
27	98	BEGIN LOOP	3
28	55	#9 TO RV	5
29	40	MIX RV	2
30	2	VORTEX ON	1
31	1	WAIT	5
32	3	VORTEX OFF	1
33	42	DRAIN RV	8
34	41	VENT RV	2
35	49	#9 RV-DRN	3
36	42	DRAIN RV	5
37	99	END LOOP	1
38	41	VENT RV	5
39	98	BEGIN LOOP	2
40	11	#9 T VB	3
41	12	#9 B VB	3

Table A-26 Flow Test 22 *(continued)*

Step#	Fxn#	Fxn Name	Time (sec)
42	9	GAS T VB	5
43	10	GAS B VB	5
44	99	END LOOP	1

Flow Test 23 (Flow Tests 19-23, module e)

Purpose: Use to determine the conductivity baseline of a solution of NMP and piperidine for *FastMoc*™ chemistry 0.25 mmol modules.

Delivery: Five samples are taken from RV and sent to conductivity flow cell.

Requirements: Standard scale (40 mL) reaction vessel (0.25 mmol), and reagents for *FastMoc*™ chemistry.

Procedure:

IMPORTANT Press the RETURN key after each change to prevent settings returning to default settings.

To download Flow Tests 19-23 from SynthAssist® software to the ABI 433A instrument:

Step	Action
1	Download Flow Tests 19-23 Chemistry file from SynthAssist® software to the ABI 433A instrument.
2	Open a new Run file in SynthAssist® software. a. Go to the **File** pull-down menu. b. Select **New**, then select **Run**.
3	Choose **Flow Test 19-23** from the top pop-up screen. Press the RETURN key to save the changes.
4	Choose **None** for sequence.
5	Type "**ee**" under Modules on the Cycles Page. Press the **RETURN** key to save the changes. **Note** Typing "ee" programs the instrument to perform the run twice.
6	Select **Save As**. a. Save the run as "Flow Test e" and add the date. b. Save the run in the Test Folder.

To send the run to the synthesizer:

Step	Action
1	**Send** the run to the ABI 433A Peptide Synthesizer. The SynthAssist® software Log window automatically appears.
2	Open the **Monitor** window. The conductivity peaks appear in the window once the run begins.
3	Place the 0.25 mmol reaction vessel, with filters, on the ABI 433 instrument.
4	Go to the "Cycle Monitor" on the ABI 433A instrument.
5	Select **No** to "Resin sampling?".

To send the run to the synthesizer: *(continued)*

Step	Action
6	Select **Yes** to “Print run events?”.
7	Select **Begin**.
8	The Log window displays the conductivity of each of five samples of the NMP/Piperidine solution. Use the value of the last peak for the conductivity baseline. a. Divide this value by ten. b. Enter it for “T” in Function 128 when it appears in the *FastMoc*™ 0.10 mmol module B-Deprotection/Ω Mon 1st Peak-X.
9	Verify similar peak height values and record the value from the second run.

Table A-27 Flow Test 23

Step#	Fxn#	Fxn Name	Time (sec)
1	1	WAIT	1
2	58	INTERRUPT	
3	135	Mon Reset	1
4	133	MonBegLoop	5
5	42	DRAIN RV	18
6	98	BEGIN LOOP	1
7	56	#10 TO RV	13
8	40	MIX RV	2
9	2	VORTEX ON	5
10	3	VORTEX OFF	1
11	42	DRAIN RV	18
12	99	END LOOP	1
13	56	#10 TO RV	12
14	79	PRS #1	10
15	51	#1 TO RV	10
16	56	#10 TO RV	4
17	40	MIX RV	2
18	2	VORTEX ON	20
19	3	VORTEX OFF	1
20	40	MIX RV	1
21	130	MonPrevPk	1
22	42	DRAIN RV	3
23	131	MonStop	1
24	132	Save Mon	1
25	134	MonEndLoop	1
26	42	DRAIN RV	18
27	98	BEGIN LOOP	3
28	55	#9 TO RV	10

Table A-27 Flow Test 23 *(continued)*

Step#	Fxn#	Fxn Name	Time (sec)
29	40	MIX RV	2
30	2	VORTEX ON	1
31	1	WAIT	5
32	3	VORTEX OFF	1
33	42	DRAIN RV	12
34	41	VENT RV	2
35	49	#9 RV-DRN	3
36	42	DRAIN RV	5
37	99	END LOOP	1
38	41	VENT RV	5
39	98	BEGIN LOOP	2
40	11	#9 T VB	3
41	12	#9 B VB	3
42	9	GAS T VB	5
43	10	GAS B VB	5
44	99	END LOOP	1

UV Fmoc-Phe Test (UV Flow Tests)

Purpose: Use to confirm Fmoc removal results in appropriate UV signal and verify the timing of solutions through the UV cell with reading UV values.

Delivery: Partial drain of a mixture of Piperidine, NMP and amino acid (Phe), from RV, through UV flowcell, with monitoring on and off for pre-set times.

Expected Results: 10 peaks in ~ 5000 to 7000 unit range, roughly equal in value

Requirements: ~100 mg Fmoc-Phe-OH in 40 mL-RV, piperidine, methanol (MeOH), and NMP.

Procedure:

UV detector should be “auto-zeroed” on NMP before beginning.

Set up a “Run” UV Flow Tests as the Chemistry:

Sequence = none; Cycle = UV Fmoc-Phe Test (e a b d)

Table A-28 UV Fmoc Phe Test

Cycle	Step #	Fxn #	Fxn Name	Time (sec)
e (MeOH, NMP to Aux)	1	1	Wait	1
	2	135	Monitoring reset	2
	3	78	Pressurize manifold	10
	4	19	#6 B VB	2
	5	101	MeOH to Aux	15
	6	1	Wait	3
	7	101	MeOH to Aux	60
	8	14	#10 B VB	2
	9	118	Flush B VB NMP	15
	10	1	Wait	3
	11	118	Flush B VB NMP	15
	12	1	Wait	3
	13	118	Flush B VB NMP	60
a (Add Pip & NMP)	1	79	PRS #1	10
	2	56	#10 B RV	45
	3	51	#1 B RV	70
	4	56	#10 B RV	78
	5	40	Mix RV	80
	6	98	Begin Loop Upper	80
	7	2	Vortex On	127
	8	3	Vortex Off	142
	9	41	Vent RV	144
	10	99	End Loop Upper	144

Table A-28 UV Fmoc Phe Test *(continued)*

Cycle	Step #	Fxn #	Fxn Name	Time (sec)
b (10 Readings)	1	1	Wait	1
	2	14	#10 B VB	2
	3	118	Flush B VB NMP	20
	4	135	Monitor Reset	2
	5	98	Begin Loop Upper	10
	6	2	Vortex On	1
	7	19	#6 B VB	2
	8	101	MeOH to Aux	20
	9	1	Wait	3
	10	14	#10 B VB	2
	11	151	Tog. User Func On	108
	12	1	Wait	20
	13	3	Vortex Off	1
	14	113	Tog. All Off	1
	15	1	Wait	2
	16	42	Drain RV	3
	17	151	Tog. User Func On	108
	18	1	Wait	5
	19	129	Monitor first peak	1
	20	1	Wait	25
	21	131	Monitor stop	1
	22	132	Read peak	1
	23	1	Wait	15
	24	113	Tog. All Off	1
	25	99	End Loop Upper	1

Table A-28 UV Fmoc Phe Test *(continued)*

Cycle	Step #	Fxn #	Fxn Name	Time (sec)
d (Drain & wash RV)	1	14	#10 B VB	2
	2	118	Flush B VB NMP	15
	3	42	Drain RV	40
	4	98	Begin Loop Upper	2
	5	56	#10 B RV	10
	6	40	Mix RV	2
	7	2	Vortex On	5
	8	3	Vortex Off	1
	9	42	Drain RV	8
	10	50	#10 RV-Drain	5
	11	42	Drain RV	8
	12	99	End Loop Upper	1
	13	98	Begin Loop Upper	1
	14	55	#9 B RV	10
	15	40	Mix RV	2
	16	2	Vortex On	3
	17	3	Vortex Off	1
	18	42	Drain RV	8
	19	49	#9 RV-Drain	5
	20	42	Drain RV	8
	21	99	End Loop Upper	1
	22	41	Vent RV	2
	23	9	Gas T VB	2
	24	10	Gas B VB	2
	25	14	#10 B VB	2
	26	118	Flush B VB NMP	15

UV Fmoc Resin Test (ecDHDC) (UV Flow Tests)

Purpose: Use to measure the UV signal-to noise values for a “clean” deprotection (resin not treated with other reagents as during coupling, no sequence-dependency).

Delivery: Piperidine/NMP to RV to remove Fmoc; subsequent rinsing, cleaning. Second attempt at Fmoc removal, as in synthetic runs, with little signal expected.

Expected Results: UV signal-to noise ~60:1 to 100:1
(background needs to be < 3.5% of first peak)

Requirements: 0.10 mole Fmoc-Gly resin (PN 401421) in 8 mL RV; piperidine, MeOH, NMP, and DCM.

Procedure:

UV detector should be “auto-zeroed” before beginning.

Set up a “Run” UV Flow Tests as the Chemistry:

Sequence = none; Cycle = UV Fmoc Resin Test (e c D H D c).

Table A-29 UV Fmoc Resin Test (ecDHDc)

Cycle	Step #	Fxn #	Fxn Name	Time (sec)
e (MeOH, NMP to Aux)	1	1	Wait	1
	2	135	Monitoring reset	2
	3	78	Pressurize manifold	10
	4	19	#6 B VB	2
	5	101	MeOH to Aux	15
	6	1	Wait	3
	7	101	MeOH to Aux	60
	8	14	#10 B VB	2
	9	118	Flush B VB NMP	15
	10	1	Wait	3
	11	118	Flush B VB NMP	15
	12	1	Wait	3
	13	118	Flush B VB NMP	60

Table A-29 UV Fmoc Resin Test (ecDHDc) *(continued)*

Cycle	Step #	Fxn #	Fxn Name	Time (sec)
c (DCM washes)	1	1	Wait	1
	2	12	#9 B VB	1
	3	9	GAS T VB	2
	4	10	GAS B VB	2
	5	98	Begin Loop Upper	6
	6	55	#9 B RV	5
	7	40	Mix RV	2
	8	2	Vortex on	1
	9	41	Vent RV	1
	10	40	Mix RV	2
	11	1	Wait	5
	12	3	Vortex off	1
	13	42	Drain RV	5
	14	41	Vent RV	2
	15	49	#9 RV-Drain	3
	16	42	Drain RV	10
	17	99	End Loop Upper	1
	18	10	GAS B VB	2
	19	41	Vent RV	2
	20	42	Drain RV	30
	21	29	#9 ACT-Drain	5
	22	22	Drain ACT	30
	23	11	#9 T VB	2
	24	12	#9 B VB	2
	25	10	GAS B VB	5
	26	9	GAS T VB	5
D (NMP washes)	1	1	Wait	1
	2	3	Vortex off	1
	3	42	Drain RV	10
	4	98	Begin Loop Upper	4
	5	41	Vent RV	2
	6	50	#10 RV-Drain	2
	7	42	Drain RV	6
	8	56	#10 B RV	4
	9	40	Mix RV	2
	10	2	Vortex on	3
	11	40	Mix RV	2
	12	3	Vortex off	1
	13	42	Drain RV	10
	14	99	End Loop Upper	1

Table A-29 UV Fmoc Resin Test (ecDHDc) *(continued)*

Cycle	Step #	Fxn #	Fxn Name	Time (sec)
H (Deprotection UV 3.5%)	1	1	Wait	1
	2	14	#10 B VB	2
	3	118	#10 B VB	20
	4	135	Monitor reset	2
	5	56	#10 B RV	4
	6	2	Vortex on	5
	7	40	Mix RV	2
	8	3	Vortex off	1
	9	42	Drain RV	8
	10	50	#10 RV-Drain	5
	11	42	Drain RV	8
	12	14	#10 B VB	2
	13	56	#10 B RV	5
	14	40	Mix RV	2
	15	79	PRS #1	10
	16	16	#1 B VB	2
	17	51	#1 B RV	5
	18	56	#10 B RV	3
	19	40	Mix RV	1
	20	2	Vortex on	1
	21	78	PRS manifold	10
	22	19	#6 B VB	2
	23	101	#6 AUX	20
	24	1	Wait	3
	25	14	#10 B VB	2
	26	151	Tog. User Func On	108
	27	1	Wait	70

Table A-29 UV Fmoc Resin Test (ecDHDc) *(continued)*

Cycle	Step #	Fxn #	Fxn Name	Time (sec)
H (Deprotection UV 3.5%), *continued*	28	3	Vortex off	1
	29	113	Tog. All Off	1
	30	1	Wait	2
	31	42	Drain RV	3
	32	151	Tog. User Func On	108
	33	1	Wait	5
	34	129	Monitor first peak	1
	35	1	Wait	25
	36	131	Monitor Stop	1
	37	132	Read Peak	1
	38	1	Wait	15
	39	113	Tog. All Off	1
	40	133	Begin Loop Monitor	3
	41	42	Drain RV	8
	42	98	Begin Loop Upper	2
	43	56	#10 B RV	5
	44	2	Vortex on	5
	45	40	Mix RV	2
	46	3	Vortex off	1
	47	42	Drain RV	8
	48	50	#10 RV-Drain	5
	49	42	Drain RV	8
	50	99	End Loop Upper	1
	51	14	#10 B VB	2
	52	56	#10 B RV	5
	53	40	Mix RV	2
	54	79	PRS #1	10
	55	16	#1 B VB	2
	56	51	#1 B RV	5
	57	56	#10 B RV	3
	58	40	Mix RV	1
	59	2	Vortex on	1
	60	78	PRS manifold	10
	61	19	#6 B VB	2
	62	101	#6 AUX	20
	63	1	Wait	3
	64	14	#10 B VB	2
	65	151	Tog. User Func On	108
	66	1	Wait	70
	67	3	Vortex off	1
	68	113	Tog. All Off	1

Table A-29 UV Fmoc Resin Test (ecDHDc) *(continued)*

Cycle	Step #	Fxn #	Fxn Name	Time (sec)
H (Deprotection UV 3.5%), *continued*	69	1	Wait	2
	70	42	Drain RV	3
	71	151	Tog. User Func On	108
	72	1	Wait	5
	73	129	Monitor first peak	1
	74	1	Wait	25
	75	131	Monitor Stop	1
	76	132	Read Peak	1
	77	1	Wait	15
	78	113	Tog. All Off	1
	79	134	End Loop Monitor	35
	80	40	Mix RV	2
	81	2	Vortex on	1
	82	14	#10 B VB	2
	83	118	#10 B VB	20
D (NMP washes)	1	1	Wait	1
	2	3	Vortex off	1
	3	42	Drain RV	10
	4	98	Begin Loop Upper	4
	5	41	Vent RV	2
	6	50	#10 RV-Drain	2
	7	42	Drain RV	6
	8	56	#10 B RV	4
	9	40	Mix RV	2
	10	2	Vortex on	3
	11	40	Mix RV	2
	12	3	Vortex off	1
	13	42	Drain RV	10
	14	99	End Loop Upper	1

Table A-29 UV Fmoc Resin Test (ecDHDc) *(continued)*

Cycle	Step #	Fxn #	Fxn Name	Time (sec)
c (DCM washes)	1	1	Wait	1
	2	12	#9 B VB	1
	3	9	GAS T VB	2
	4	10	GAS B VB	2
	5	98	Begin Loop Upper	6
	6	55	#9 B RV	5
	7	40	Mix RV	2
	8	2	Vortex on	1
	9	41	Vent RV	1
	10	40	Mix RV	2
	11	1	Wait	5
	12	3	Vortex off	1
	13	42	Drain RV	5
	14	41	Vent RV	2
	15	49	#9 RV-Drain	3
	16	42	Drain RV	10
	17	99	End Loop Upper	1
	18	10	GAS B VB	2
	19	41	Vent RV	2
	20	42	Drain RV	30
	21	29	#9 ACT-Drain	5
	22	22	Drain ACT	30
	23	11	#9 T VB	2
	24	12	#9 B VB	2
	25	10	GAS B VB	5
	26	9	GAS T VB	5

Metering Loop

B

Calibrating the Metering Loop

How to Calibrate the Metering Loop

The following procedure is necessary only if the metering loop is replaced or out of calibration.

To calibrate the metering loop:

Step	Action
1	Place 100% NMP in bottle 7.
2	Calibrate the 0.50-mL metering loop using Flow Tests 17 (bottle 7). a. Unscrew the fitting from the cartridge inline filter. b. Preweigh a dry, empty cartridge with the cap removed, and hold it so that the fitting from valve 12 is just inside. c. Run Flow Test 17. Weight of the delivery could be substantially greater than the specification because the line is provided with excess length for cutting to size for each instrument.
3	To shorten the loop, remove the line at valve 13.
4	Push the fitting and ferrule back up the line.
5	Carefully cut the line with a sharp razor blade. **Note** Be sure the cut end is perpendicular to the rest of the tube. **Note** The volume is roughly equivalent to 0.03 g NMP per inch of line.
6	Repeat steps 2 and 3 until the delivery is within the specification.
7	Reconnect the line from valve 12 to the cartridge inline filter.

Laboratory Ventilation Requirements

C

Waste/Exhaust System and System Vent

Overview

The ABI 433A Peptide Synthesizer has one system for removing gaseous wastes. The waste/exhaust system removes both fluid and gaseous waste from the instrument's fluid lines. The fluid wastes accumulate in waste a container, and the gaseous waste is delivered via an exhaust line to the laboratory ventilation system.

Laboratory Ventilation Specifications

The laboratory ventilation system(s) to this instrument must be operating properly whenever the instrument power is on, waste is in the waste container, or reagents are on the instrument.

For the ABI 433A Peptide Synthesizer, proper ventilation operation specifications include:

- A duct or fume hood with an average of 30 linear m/min (100 linear ft/min) face-level velocity of airflow. The minimum velocity at any point in the hood is 24 linear m/min (80 linear ft/min), and the maximum velocity is 38 linear m/min (125 linear ft/min).
- Use a duct system that allows vapor or gas movement of 1000 to 2000 ft/min (305 to 610 m/min).

About the Waste/Exhaust System

The waste/exhaust system is composed of a fluid waste line and a gaseous waste exhaust line. One end of each line is attached to a cap fitted to a 9.5-L (2.5-gal) polyethylene bottle (carboy) which must be placed in a secondary container. The other end of the fluid waste line is attached to a waste port on the back of the instrument.The gaseous waste exhaust line has a 9.525-mm outer diameter (3/8-in. o.d.). and conducts the waste fumes and gasses that enter the bottle to a fume hood or duct system for disposal.

⚠ WARNING CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Potential Exposure to Hazardous Waste

Always wear personal protection equipment such as a laboratory coat, eye protection, and gloves that resist waste chemicals when you work on waste bottles, exhaust line tubing, and liquid traps.

Connecting the Gaseous Waste Exhaust Line

Follow these guidelines when connecting the tubing from the instrument's gaseous waste exhaust to the ventilation system:

- Use the shortest possible length and the straightest possible run of polypropylene tubing. Tubing length should not exceed 4.5 m (15 ft).
- Make sure that the tubing does not have low points that can trap residue or condensation.
- Fasten the tubing securely. Use polypropylene or Teflon fasteners. Do not use brass; it corrodes. Be careful not to puncture tubing.
- Place the tubing away from sources of potential damage, such as heat, flame, or points of contact (rubbing) with other objects.
- Place the tubing end as far as possible into the duct, canopy, or hood.
- Oncoming air movement through the duct or canopy should not face the open end of the tubing.

Connecting the Fluid Waste Line

Connect the fluid waste line from the instrument to the waste bottle so that it drops vertically. Doing so prevents liquid and waste from accumulating and blocking the flow.

Connecting the Flexible Duct

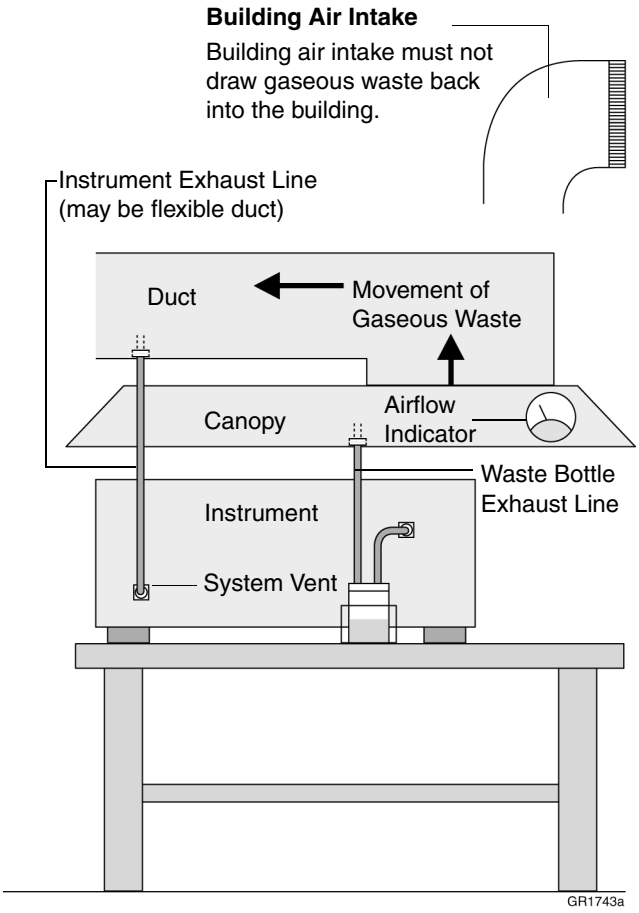
Follow these guidelines in connecting the flexible duct from the instrument's system vent to the
laboratory ventilation system.

- Use the shortest possible length and straightest possible run of flexible duct.
- Make sure the flexible duct does not have low points that can trap residue or condensation.
- Keep the flexible duct away from sources of potential damage, such as contact, heat, or flame.
- Fasten the flexible duct securely to the ventilation duct; use the clamp(s) provided with the instrument.

Heat Production

The thermal output of the ABI 433A instrument is 3.4 Btu/h (1 W). Consult the facilities department regarding ventilation requirements for this level of heat output.

Laboratory Ventilation System: Canopy and/or Duct



Duct-Work

- Verify that ducts are constructed of materials that are compatible with vented waste.
- Do not simultaneously vent strong oxidizers, bases or other chemicals that are incompatible with gaseous waste.
- Maintain negative pressure in duct.

Waste Line Tubing (or flexible duct)

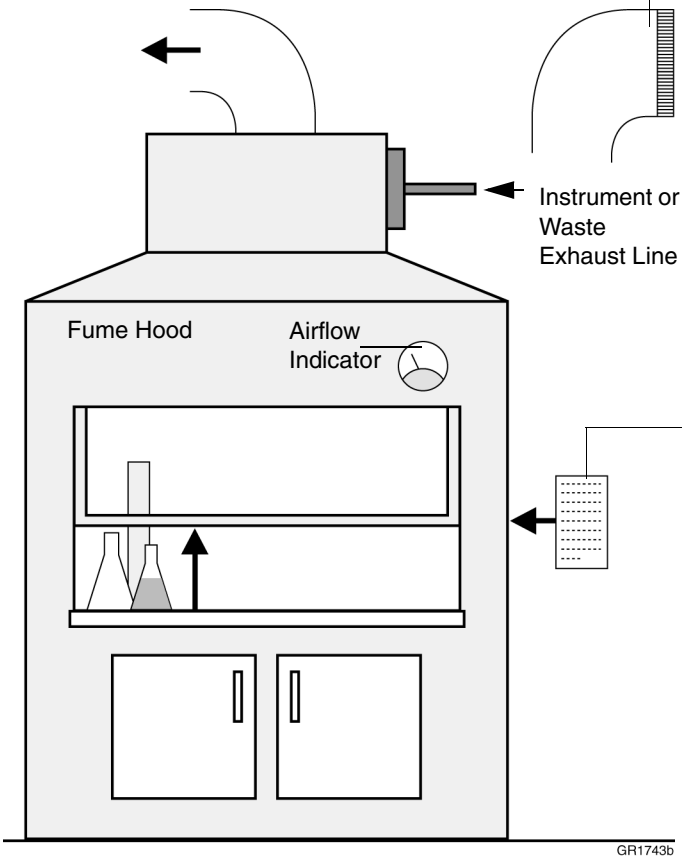
- Face open end of tubing away from incoming air.
- Place tubing as far into the hood or canopy as possible.
- Use a straight-upward run of no more than 3 m (10 ft) of polypropylene tubing.
- Securely fasten tubing with polypropylene or Teflon fasteners, not brass (brass corrodes).

Ventilation Operation

- Operate the laboratory ventilation system whenever the instrument power is on, waste is in the waste bottle, or reagents are on the instrument. This includes nights and weekends.
- Do not connect the instrument to a ductless hood or a system that purifies/filters air and returns it to the room.

Figure C-1 Venting gaseous waste directly through connection to a duct

Laboratory Ventilation System: Fume Hood


Building Air Intake
Building air intake must not draw gaseous waste back into the building.
Instrument or Waste Exhaust Line
Fume Hood
Airflow Indicator
GR1743b

Fume Hood

- Verify that construction materials are compatible with vented waste.
- Do not simultaneously vent strong oxidizers, bases or other chemicals that are incompatible with gaseous waste.
- Locate hood away from air currents created by air-conditioning ducts, fans, windows, doors, or moving objects or people.
- Verify that hood meets local, state/provincial, or national standards.
- Post label at sash level that provides an average face-level air-flow velocity of 30 linear m/min (100 linear ft/min). See "Laboratory Ventilation Specifications" on page C-1.

Waste Line Tubing

- Use no more than 4.5 m (15 ft) of polypropylene tubing.
- Securely fasten tubing with polypropylene or Teflon fasteners, not brass (brass corrodes).

Ventilation Operation

- Operate the laboratory ventilation system whenever the instrument power is on, waste is in the waste bottle, or reagents are on the instrument. This includes nights and weekends.
- Do not connect the instrument to a ductless hood or a system that purifies/filters air and returns it to the room.

Figure C-2 Venting gaseous waste produced by an instrument to a fume hood

Fume Hood Operation

Follow these guidelines for fume hood operation and maintenance:

- Operate the fume hood continuously, including nights and weekends.
- Operate the fume hood whenever the instrument power is on, waste is in the waste container, or reagents are on the instrument.
- Use a fume hood that is constructed of materials that are compatible with the waste materials/chemicals being generated or exhausted.
- Locate the fume hood away from air currents generated by air conditioning ducts, fans, windows, doors, and moving equipment and persons.
- Locate the fume hood exhaust vent where gaseous waste cannot be drawn back into the building.
- Affix a sign or label to indicate the position of the fume hood sash that produces an average airflow of 100 linear ft/min face velocity. The minimum flow velocity at any point in the hood is 80 linear ft/min, and the maximum is 125 linear ft/min.
- Ensure that the fume hood meets all local, state/provincial, or national safety requirements.
- Have a safety professional or mechanical ventilation expert check and record air velocity a minimum of once a year.
- Inspect and maintain the exhaust system, including fans and motors, a minimum of once a year.

Duct System Operation

Follow these guidelines for duct system operation and maintenance:

- Operate the fume hood continuously, including nights and weekends.
- Operate the duct system whenever the instrument power is on, waste is in the waste container, or reagents are on the instrument.
- Use a duct system constructed of PVDF tubing or other materials compatible with the waste material being generated.
- Do not allow the duct system to come into contact with strong oxidizers, bases, or other chemicals that are incompatible with gaseous waste.
- Use a duct system that allows vapor or gas movement of 1000 to 2000 ft/min (305 to 610 m/min).

Waste Ventilation Warnings

⚠ WARNING CHEMICAL WASTE HAZARD. Waste produced by Applied Biosystems instruments can be hazardous and can cause injury, illness, or death.

- Operate a vented instrument only if it is connected in accordance with all requirements.
- Operate the system venting gaseous waste whenever the instrument power is on, waste is in the waste container, or reagents are on the instrument.
- Handle all liquid, solid, and gaseous waste as potentially hazardous.
- See the MSDSs provided by the manufacturer(s) of chemicals used at your site for more information.
- Connect the exhaust line so that it leads to the fume hood or duct in a straight and upward direction. Low points will allow condensation to collect, preventing flow through the instrument.
- Do not connect the waste vent line to a ductless hood or to a system that purifies and filters air and returns it to the room.
- Always mix and prepare hazardous materials beneath an operating fume hood.
- Dispose of waste in accordance with all local, state/provincial, or national health and safety regulations and laws.
- Venting hazardous waste may require local, state/provincial, or national air permits.

Routine Maintenance Tasks

- Check and record face velocity periodically (at least twice a year). If results fail to meet standards, make ventilation adjustments as soon as possible to prevent employee exposure to hazardous fumes.
- Inspect and maintain exhaust system periodically (at least once a year), including fans and motors.
- Visually inspect tubing periodically for breakage, crimping, corrosion (if metal), or other damage. Replace the tubing as necessary.
- Flush the vent line with pure, dry pressurized gas to remove buildup of condensation or particulates. Handle and dispose of all wastes as if they were hazardous.

Troubleshooting the UV FastMoc™ Kit

D

Troubleshooting

Where to Look

Refer to the troubleshooting section in the *PerkinElmer Series 200 UV/Vis Detector User Manual* (Rev A) for most information.

Absorbance Value Error

The table below describes an absorbance value reading error, its possible causes, and possible solutions.

Note The following information is not documented in the current version of the *PerkinElmer Series 200 UV/Vis Detector User Manual* (Rev A).

Error Message	Possible Cause	Possible Solution
Absorbance value reading shows as ERR! or blinking	♦ The error is caused by the absorbance reading being <-0.05. ♦ This error may occur during warm up when the lamp has not yet stabilized.	♦ Normally all operating absorbencies are easily within this range and the error does not appear.
	♦ Should the abs drift to < -0.05, the error would show up at other times as well.	♦ Pressing autozero on the keypad should correct the problem and restore the Absorbance display.
	♦ The conditions for the error to occur are either the absorbance1 is lower than -0.05 or higher than +1.49.	♦ If the error appears frequently there is a drift problem that will need to be resolved.
NMP absorbance is less than zero.	♦ Using old Methyl Alcohol (MEOH) to zero the S200	♦ Install a fresh bottle of MEOH at position 6. Prime via Fxn 19, then run Fxn 101 ~ 60 seconds. Re-zero the S200 detector and then test NMP absorbance. Also, run Fxn 118.

Note UV data to the 433A is not being truncated or lost when the S200 absorbance display blinks or shows ERR!

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